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MEETING ABSTRACTS

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P01 Reproductive Genetics/Prenatal Genetics

P01.01A
Internal analytical verification of a targeted microarray-based cell-free DNA test for 22q11.2 deletion

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Objectives: Laboratories are required to verify their assays determining that the test is being performed correctly, even if kit/software are CE-IVD marked with suitable performance specifications or a new test is implemented using a technology that is already well established in a source/reference laboratory. This study presents the internal analytical verification after the implementation of 22q11.2DS cfDNA test by a targeted microarray-based technology in an independent decentralized European laboratory.

Methods: Analytical sensitivity: 25 samples with 22q11.2DS were tested (2 maternal plasma and 23 simulated pregnancy samples). Deletions spanned through the A-D 22q11.2 LCRs, sizes ranged from 2.37 and 2.89Mb and simulated fetal fractions ranged from 7 to 39%. Analytical specificity: 423 prospectively ascertained maternal plasma samples with no known diagnosis of fetal/maternal 22q11.2DS were tested. This study was approved by the laboratory IRB. All samples were de-identified before study.

Results: Analytical sensitivity was 96.00%(95%CI:80.46-99.28) (24/25) and specificity was 99.76%(95%CI:98.67-99.96) (422/423) not statistically different from that reported in a previous clinical validation/verification (OR 0.12;95% CI:0.01-0.97 and OR 0.54;95%CI:0.07-4.31). The specificity must be considered a lower-bound estimate as the sample classified as "false positives" may be a true positive from an undiagnosed mother or affected fetus.

Conclusions: We have verified the internal analytical performances of a targeted microarray-based cfDNA test for 22q11.2 deletions inside the typical 3Mb region in the decentralized laboratory match the performance specification of the source laboratory. The low FPR, below 0.5%, for this cfDNA test expansion is critical when testing low-risk population as it highly impacts PPV.

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P01.013C

Potential role of *ACKR3/CXCR7* duplication in pregnancy loss

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Introduction: The atypical chemokine receptor 3 (ACKR3) is highly expressed in vascularized structures including the placenta and umbilical cord. Aberrant expression of ACKR3/ligands resulting in dysregulation of trophoblast-endometrial interaction has been implicated in recurrent pregnancy loss, intrauterine growth restriction, preterm labor, and preeclampsia. Copy number variations (CNV's) overlapping with *ACKR3* have been documented in patients with variable findings including neurodevelopmental defects (NDD's), and in 0.01% of the healthy population (DGV). No CNV's involving *ACKR3* have been reported in pregnancy loss. Here we present our novel data from our retrospective chromosome microarray analysis (CMA) associating duplications of *ACKR3* with spontaneous abortions (SAB's) and intrauterine fetal demise (IUFD).

Materials and Methods: Retrospective CMA was performed from ~4,700 samples to identify CNV's overlapping with *ACKR3* (aka *CXCR7*; 2q37.3), of which ~350 were products of conception (POC).

Results: This study revealed 11 CNV's overlapping with *ACKR3*. Nine gains were detected from six SAB's and three IUFD (7 males, 2 females) ranging from small intervals (n = 4, 36.8-65.5 kb) to trisomy 2 (n = 4) and tetrasomy 2 (n = 1). These gains account for 0.19% of all CMA cases and 2.6% of all POC cases. A 610 kb gain and a 6.1 Mb loss were observed from blood samples of two patients with NDD's.

Conclusions: This study is the first to report rare duplications of *ACKR3* in SAB's and IUFD, providing additional information to existing genomics data. Findings from this study may aid in enhanced understanding of *ACKR3*'s role in pregnancy, genetic counseling and management of pregnancy loss.

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P01.05A

Aneuploidy rate in 530 miscarriage cases

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Introduction: Spontaneous loss of pregnancy before the fetus reaches viability is the most frequent pregnancy complication. The term miscarriage includes all pregnancy losses from the time of conception until 20 weeks of gestation. According to published data about 50% of first-trimester pregnancy losses are the consequence of fetal chromosomal abnormalities. Most of these abnormalities are numerical (86%).

Materials and Methods: A total of 570 spontaneous miscarriage cases were collected between 2002 and 2017, referred to our laboratory from all over the country. DNA was extracted from placental or fetal tissue. Samples that did not contain tissue, appropriate for DNA analysis (7%) were excluded. QF-PCR focused on chromosomes 13, 18, 21, X and Y was performed on 530 samples. One hundred of them were additionally analyzed for aneuploidies involving chromosomes 15, 16 and 22.

Results: Aneuploidy was found in 117 (22% of the cases). Thirty-five of them (29.9%) were with triploidy, 27 (23%) - with trisomy 21, 24 (20%) - with monosomy X, 17 (14%) - with trisomy 13 and 14(11.9%) - with trisomy 18. Additionally, trisomy 15 was found in 2 cases, trisomy 16 - in 4 and trisomy 22 in 3 cases out from 100.

Conclusions: QF-PCR analysis, being rapid and cost-efficient proved to be useful for genetic analysis of miscarriage samples. Although the vast majority of chromosomal aneuploidies in miscarriages are de novo, such results provide valuable information for the clinicians and genetic counselors and for the couple.

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P01.06B

Clinical implementation of a custom oligonucleotide array-CGH. Experience in the largest cohort of Spanish prenatal samples (>3400 samples) Clinical implementation of a custom oligonucleotide array-CGH. Experience in the largest cohort of Spanish prenatal samples (&gt3400 samples)

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Introduction: The implementation of genomic array in prenatal routines, when accompanied by pre- and post-test genetic counselling, has demonstrated its utility by fulfilling the longstanding need for a diagnostic test with a higher resolution and higher diagnostic yield than its predecessor, the conventional karyotype.

Materials and methods: Array CGH was performed in 3.438 prenatal samples (2.604 amniotic fluids, 728 corions and 106 fetal samples), using a custom 60K oligonucleotide-based microarray (qChip® CM) designed to maximize the detection of clinically relevant copy-number alterations, and minimize the detection of variants of unknown significance (VOUS). As a general rule, VOUS with unclear phenotypic effect according to current knowledge, and some susceptibility variants are not reported.

Results: We identified a total of 247 pathogenic or probably pathogenic alterations (detection rate: 7.18%) and 60 VOUS (1.74%). As expected, the greatest pathogenic detection rate (7.66%, 202/2637) was among fetuses with ultrasound anomalies, while detection rate was 5.61% (45/801) in ecografically normal gestations (2.24%, 18/801 only with altered maternal serum screning). The vast majority of the VOUS were inherited from a non-affected parent (89.65%) and could be reclassified as most likely benign.

Conclusions: Our series reinforces the clinical utility of prenatal microarray testing: it nearly doubles the diagnostic yield of conventional karyotype (110/247 with variants <10Mb), with no significant increase in the frequency of VOUS that could interfere in decision making. In our experience, we highlight the importance of implementing aCGH in prenatal routines (for all gestations with an indication of invasive fetal sampling).

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P01.07C

Targeted exome sequencing for mutation detection in rare autosomal recessive disorders in families with recurrent undiagnosed fetal anomalies

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Introduction: Prenatal ultrasound allows the detection of fetal malformation syndromes which often remain without conclusive diagnosis. In case of a recurrent fetal phenotype an autosomal recessive disorder is suspected.

Materials and Methods: We report on 2 families with recurrent fetal anomalies:

-Family A: Consanguineous couple from Afghanistan, who experienced 2 terminated pregnancies due to severe brain malformation with hydrocephalus and absence of cerebellar vermis.
-Family B: Non-consanguineous couple from Kosovo who experienced 2 perinatal deaths after pregnancies marked by third trimester polyhydramnios and fetal akinesia. Both newborns presented at post-mortem examination distal joints flexion contractures.

We performed on stored fetal DNA whole exome sequencing (WES) with targeted bioinformatic analysis. In family A, we analyzed genes associated with brain malformation and in family B genes known to cause arthrogryposis and fetal akinesia.

Results: In family A, we found a novel nonsense homozygous mutation in *LARGE1* causing a rare form of congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies (OMIM: 613154) and in family B a novel nonsense homozygous mutation in *GLDN* causing lethal congenital contracture syndrome 11 (OMIM: 617194). In both families, Sanger sequencing confirmed homozygosity in the proband and in the second affected fetus, and also confirmed carrier status in both parents. Based on these results, we were able to offer invasive prenatal testing for both families.

Conclusions: Targeted WES is an effective tool for mutation detection in rare autosomal recessive disorders causing recurrent undiagnosed fetal phenotypes, allowing accurate recurrence risk counseling and early prenatal diagnosis for future pregnancies.

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P01.08D

Whole exome sequencing in non-obstructive azoospermia allows the identification of a high-risk subgroup of infertile men for undiagnosed Fanconi Anemia, a cancer-prone disease

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Background: The etiology of non-obstructive azoospermia (NOA) remains unknown in about 40% of cases and a genetic origin is likely to be involved in idiopathic NOA. Genes implicated in stem cell proliferation and DNA repair may cause isolated NOA or be responsible for syndromic diseases, such as Fanconi Anemia (FA). In about 10% of FA cases the diagnosis is delayed until adulthood when a malignant tumor is diagnosed.

Methods: Whole-Exome Sequencing (WES) in an idiopathic NOA patient (index case) with consanguineous parents. Sanger sequencing of the *FANCA* gene in the brother of the index case and in 27 selected NOA patients. DEB-induced chromosome breakage test.

Results: a rare pathogenic homozygous *FANCA* variant (c.2639G>A) was identified in the index case, affected by Sertoli Cell only syndrome. The patient's brother (also with NOA) carried the same genotype. The two brothers did not manifest overt anemia, though chromosomal breakage test confirmed FA. In 27 selected NOA patients with similar testicular phenotype and borderline/mild hematological alterations revealed one additional SCOS patient with compound heterozygosis in *FANCA* (c.3788_3790delTCT;c.3913C>T).

Conclusions: we identified a specific subgroup of NOA patients with mild or borderline hematological alterations presenting high frequency of occult FA (7.1%). This discovery have important clinical implications: the screening for *FANCA* mutations in such patients may allow the identification of undiagnosed FA; it corroborates previous epidemiological observations reporting a higher risk of morbidity (including cancer) and a lower life expectancy in infertile men in respect to fertile, normozoospermic men.

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P01.09A

Antenatal presentation of Bardet-Biedl syndrome: the question of phenotype-genotype correlations

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Introduction: Bardet-Biedl syndrome (BBS) is an emblematic ciliopathy associating retinal dystrophy, obesity, postaxial polydactyly, learning disabilities and renal dysfunction. Before birth, enlarged/cystic kidneys as well as polydactyly revealed by ultrasound (US) are the usual hints to consider this diagnosis in absence of familial history. However, these symptoms are not specific of BBS, raising the problem of differential diagnoses and prognosis. Molecular diagnosis during pregnancies remains a timely challenge for this heterogeneous disease (21 known BBS genes). We report a large cohort of BBS fetuses to better characterize antenatal phenotype-genotype correlations.

Materials and Methods: Prenatal US and/or autopsic data from 74 interrupted fetuses with putative BBS diagnosis were collected.

Results: Using targeted Next Generation Sequencing, we established a molecular diagnostic in 52 cases mainly in BBS genes (47 cases) following the classical gene distribution, but also in other ciliopathy genes (5 cases). Polydactyly (81%, of postaxial localization only) and renal cysts (72%) were the most prevalent symptoms in BBS-mutated fetuses. However, autopsy revealed polydactyly missed by US in 44% of cases. Hydrometrocolpos, evocative of BBS, was found in 3 cases. Ductal plate anomalies, hepatic portal fibrosis, cardiovascular or central nervous system anomalies were rare (6, 4 and 6 cases respectively).

Conclusion: Polydactyly and renal anomalies are confirmed as major prenatal manifestations for BBS. Polydactyly must be carefully controlled in case of apparent isolated renal anomalies. The use of prenatal "fast track" NGS in case of enlarged/cystic kidneys and/or polydactyly has a high utility for diagnosis and prognosis for improved parental information.

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P01.10B

Different pattern of concordance rate in birth defects according to the zygosity in twins

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Objective: The pathogenesis of birth defects is multifactorial, and comparing the concordance rate of birth defects according to zygosity in the twin can help to understand the genetic and environmental impacts of the occurrence of birth defects. The objective of this study was to determine the concordance rate of birth defects in central nervous system (CNS) and cardiovascular system (CV), according to the zygosity.

Method: Twins born at Seoul National University Hospital were examined. Zygosity was confirmed by sex, chorionicity, and DNA analysis of umbilical cord blood. PCR amplified short tandem repeat (STR analysis) was conducted with the DNA of cord blood.

Result: The risk of birth defects in CNS and CV of the second twin (F2) was increased when birth defects was present in the first twin (F1) (table). However, higher concordance rates of CNS birth defects were observed in MZ than in DZ [probandwise concordance rate (%, 95% CI): 50.00 (15.70-84.30) in MZ vs. 0.00 (0.00-23.16) in DZ, $p < 0.01$], whereas the concordance rate of CV birth defects was not different [probandwise concordance rate (%, 95% CI): 26.67 (14.60-41.94) in MZ vs. 20.29 (11.56-31.69) in DZ, p = NS]

Conclusion: The concordance rate according to the zygosity was different between CNS and CV birth defects. It may be speculated that CNS birth defects are highly genetically affected, whereas CV birth defects are affected by the environment.

Birth defects in the 2nd twin

	Birth defects in the 1st twin (-)	Birth defects in the 1st twin (+)	*p*-value
Central nervous system	**0.5% (12/2375)**	**25% (2/8)**	**<0.05**
- Monozygotic	0.4% (3/681)	66.7% (2/3)	<0.001
- Dizygotic	0.5% (9/1694)	0% (0/5)	NS
Cardiovascular system	**1.9% (45/2327)**	**23.2% (13/56)**	**<0.001**
- Monozygotic	2.4% (16/661)	26.1% (6/23)	<0.001
- Dizygotic	1.7% (29/1666)	21.2% (7/33)	<0.001

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P01.11C

Dizygotic sex-discordant IVF/ICSI twins with blood but not tissue chimerism

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Introduction: Blood-chimerism in dizigotic twins is very rare condition with presence of two genetically distinct cell lines in one individual that are derived from two separate zygotes. It can occur through intrauterine transfer of hematopoietic cells between the fetuses via vascular anastomoses. Here we present a case of IVF/ICSI pregnancy initially defined as monozygotic; during second trimester a sex-discordance between twins was noted.

Materials and Methods: Cytogenetic analysis of whole blood lymphocytes was performed twice - after delivery and at age of 6 months of the children. DNA was subsequently extracted from buccal cells and from suspensions of cultivated lymphocytes used for karyotyping. DNA profiling was performed with a special attention on markers located on X and Y-chromosomes.

Results: A demonstrable blood chimerism was detected by karioyping and DNA analysis after birth in both twins. Karyotypes for twin 1 (healthy female) and twin 2 (healthy male) at time of birth were chi46,XY[78]/46,XX[22] and chi46,XY[69]/46,XX[31], respectively. After 6 months the karyotypes detected were chi46,XY[87]/46,XX[13] and chi46,XY[86]/46,XX[14], respectively. DNA data from cultivated lymphocytes was in accordance with the cytogenetic results and confined the blood chimerism. This phenomenon was not detected by DNA analysis of buccal cells of both twins.

Conclusions: An efficient use of both cytogenetic and molecular analysis techniques in this case of blood but not buccal cells chimerism was demonstrated. When XX/XY chimerism is detected in blood cells, a careful monitoring of reproductive organs of the twins is recommended. In this case no genital anomalies are detected up to now.

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P01.12D

Comprehensive Carrier Screening using a combination of NGS panel, ddPCR and RPA

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Introduction: Carrier screening is a genetic test used to determine if a healthy person is a carrier of a recessive genetic disease. The goal of carrier screening is to help individuals understand their risks of having a child with a genetic disorder and review the range of options available to guide pregnancy and family planning.

Method & test design rationale: Autosomal and X-linked recessive disorders were selected based on the following criteria: Early onset and high-severity disorders, high carrier frequency, availability of treatment and effect on quality of life. Based on this, a NGS panel of 331 genes was designed assessing CCDS +/-20 bases and relevant deep intronic mutations from HGMD® and Centogene's proprietary variant database CentoMD®. An in-house developed pipeline provides CNV calling on NGS data. Technically challenging but relevant risk genes (FMR1, SMN1, CYP21A2) are analyzed by additional assays based on ddPCR, RPA and Sanger. Adult-onset conditions and X-linked genes in males are not analyzed.

Results: Routine NGS processing covers ≥99% of targeted bases at ≥20 reads. For a comprehensive carrier evaluation, "pathogenic", "likely pathogenic" and strong VUS (class 3.1) are reported. Couples are offered complete screening for partner one and check for partner two genes with actionable variants.

Conclusions: CentoScreen® offers a comprehensive carrier screening to accurately determine genetic risks. It can be especially used in couples from regions with high consanguinity as well as ethnicities with high incidence of certain genetic diseases even without any family history of genetic disease to understand their genetic risks.

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P01.13A

CarrierTest - one year experience with expanded preconception carrier screening

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We have designed a NGS (next-generation sequencing) amplicon-based panel testing 835 key mutations of 77 genes which can influence reproductive health of prospective parents or can cause recessive disorders in offspring. We have developed a bioinformatic pipeline using local installation of Ensembl genomic database for annotation and SQL server variant database for data handling and clinical reporting. To replace MLPA and fragmentation analysis methods we developed coverage analysis-based CNV detection of frequent large deletions of *SMN1* and *CFTR* genes. A software tool developed for the application generates report semi-automatically. Results are grouped according to clinical impact: (1) mutations in genes associated with severe recessive disorders in offspring (e.g. *SMN1, CFTR, GJB2* genes), (2) mutations in set of genes predisposing to blood hypercoagulation- trombophilic profile (*F2, F5, MTHFR, ANXA5*- M2 haplotype), (3) ovarian response to FSH (*FSHR* polymorphism).

We analysed 3238 samples In the year 2017: 1296 couples in IVF programme (2592 individuals), 354 gamete donors (F 339, M 15) and 141 individuals with a reproductive disorder without compatibility testing (F 88, M 53). The most frequent occurrence of carriers was recorded in the commonly investigated genes (*SMN1, CFTR, GJB2*), but also in other genes (e.g. *BTD, MEFV, ABCA4, SERPINA1, ACADS, DHCR7, PAH, AR*). 28 couples with reproductive risks were identified, who were offered preimplantation genetic testing of monogenic diseases (PGT-M). So far we have done IVF with PGT-M in four cases (*AR, ABCA4, CFTR* genes) and invasive prenatal diagnosis in two cases after a spontaneous conception (*SERPINA1* and *PAH* genes).

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P01.14B

Male infertility and deafness caused by homozygous deletion of CATSPER2 and STRC on 15q15.3

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We report on three brothers and one sister born to non-consanguineous parents. The sister and two of the brothers suffer from hearing loss. In addition, the affected brothers, but not the sister, are infertile. Although semen analyses yielded normal sperm concentrations, motility, and morphology (= normozoospermia), the sperm failed to fertilize the oocytes in vitro. However, both brothers conceived a child after ICSI. The sister and the unaffected brother each conceived children spontaneously. Conventional chromosomal analysis of the affected brothers demonstrated apparently normal karyotypes, whereas array-CGH revealed a homozygous loss of approximately 45 kb on chromosome 15q15.3. Homozygous microdeletions in 15q15.3 lead to the deafness-infertility syndrome (OMIM 611102) that is characterized by prelingual hearing loss and infertility in males but not in females. This homozygous loss was also demonstrated in the sister. Both parents were heterozygous for this deletion. The deletion encompasses the genes CKMT1B, STRC and CATSPER2. CKMT1B is translated to a mitochondrial creatine kinase. STRC encodes stereocilin, a protein required for the function ofthe outer hair cells in the inner ear. CATSPER2 encodes a subunit of the sperm-specific CatSper Ca^{2+}-channel complex. CatSper controls the intracellular Ca^{2+} concentration and, thereby, the swimming behavior of sperm. Functional analysis of sperm from one of the affected brothers demonstrated a lack of functional CatSper channels - this deficit explains the infertility and IVF failure (Brenker et al., 2018 https://doi.org/10.1073/pnas.1717929115). This work was carried out within the frame of the DFG Clinical Research Unit 'Male Germ Cells: from Genes to Function' (CRU 326).

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P01.15C

Positive predictive value (PPV) estimates for cell-free DNA based screening and choice of confirmatory invasive procedure: experience of a large Italian referral prenatal diagnostic laboratory

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Introduction: The aim of this study is to determine the PPV for common trisomies and SCAs using cfDNA screening, based on karyotype results obtained following a high-risk cfDNA testing result.

Materials and Methods: Initial CfDNA screening was ordered by the referring physicians and performed by a variety of technologies. One-hundreds and eighteen confirmatory samples were sent to our lab following a high risk cfDNA test result. PPV stratified by maternal age was calculated for maternal age-dependent aneuploidies.

Results: PPV for T13, T18, T21 was 23.1%, 66.7%, 91.9%, respectively. PPV for SCAs was 7.7% for 45,X, 50% for 47,XXX, 30.8% for 47,XXY and 50% for 47, XYY. In women <35y and ≥35y the PPV for T21, T18 and T13 was, 92%(81,97)*, 57%(25,84)*, 33.3%(12,65)*, and 90(60,98)*, 100%(34,100)*, 0%(0,49)*, respectively. Most of prenatal confirmations were performed on amniocytes (81/116;69.8%); this trend was more evident when the high-risk result involved a SCA (28/33;84.8%) versus a trisomy (54/84;64.3%) (OR: 3.1111;95%CI: 1.1-8.9). However, CVS shows a higher PPV (28/30,93.3%) for non-mosaic trisomies than AF (37/54,68.5%) (OR: 6.43;95%CI: 1.4-30.2).

Conclusions: The higher rate of confirmatory AF after a SCA high-risk result probably indicates that referring clinicians are aware of the proneness of sex chromosomes to generate confined placental mosaicism, therefore causing a decreased PPV when using CVS for SCAs. A possible hypothesis for the higher PPV of CVS for non-mosaic trisomies might be the performance of an ultrasound-scan before the choice of confirmatory invasive procedure, whereby amniocentesis is preferred when no ultrasound anomalies are identified. * 95th% CI

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P01.16D

Genome-wide cell-free fetal DNA screening in routine non-invasive prenatal testing practice: a prospective study on over 38.000 clinical cases

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Introduction: Conventional cell-free fetal DNA (cfDNA)-based non-invasive prenatal testing (NIPT) focuses on detection of common aneuploidies, leaving a gap of ~17% of clinically relevant chromosomal abnormalities that would go undetected. Genome-wide NIPT would greatly expand the range of chromosomal rearrangements detectable. In this study, we expanded conventional cfDNA-based NIPT to cover the entire genome in a large general population of pregnant women, in order to assess the incidence of chromosomal abnormalities not detectable by traditional NIPT.

Method:38.095 pregnant women undergoing genome-wide cfDNA-based NIPT were enrolled in the study. Sequencing data were analyzed using algorithms for common fetal aneuploidies, aneuploidies and subchromosomal aberrations. Clinical outcomes were obtained in 37.804 pregnancies.

Results:Clinically relevant chromosomal abnormalities were detected in 630 (1.7%) pregnancies, 560 (1.6%) of which were confirmed by invasive prenatal diagnosis. In 500/560 cases common aneuploidies were involved, 30/560 were rare autosomal trisomies (RAT) and 30/560 were segmental imbalances. 60 fetal conditions would have otherwise been overlooked if only a conventional NIPT had been performed. The specificity for common aneuploidy, RAT and segmental aneuploidies was 99.92%, 99.98%, and 99.98%, respectively; the sensitivity was 100%.

Conclusion: The results of this study demonstrate that genome-wide cfDNA analysis represents an enhanced screening tool for prenatal detection of chromosomal abnormalities, allowing identification of clinically relevant imbalances that are not detectable by conventional cfDNA testing. This screening provides improved detection rate as compared to conventional NIPT, with no appreciable decrease in specificity. These findings provide substantial evidence for the feasibility of introducing genome-wide NIPT into routine prenatal diagnosis practice.

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P01.17A

Microarray analysis in fetuses with a high risk combined test

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Introduction: to investigate the incremental yield of detecting submicroscopic chromosomal abnormalities by genomic microarray compared to karyotyping in high risk fetuses after combined testing.

Materials and Methods: A total of 250 fetuses, with a high risk after combined test, were tested by conventional CVS karyotyping. If the short-term cytogenetic analysis appeared to be normal, women were informed about CGH-array analysis. If the woman refused the CGH-array, a karyotype analysis was carried out using the long-term culture method.

Results: chromosomal abnormalities were detected in 58 fetuses (23%), particularly in pregnancies complicated by ultrasound abnormalities. In 192 fetuses (77%), the karyotype, after short-term analysis, was normal. All these women were counselled: 74% agreed to proceed with CGH-array analysis while26% refused. In these cases, karyotyping was completed with long-term culture methods confirming the chromosomal normality. Only 19% of the women with a fetus with an increased NT (> 3.5 mm) or ultrasound abnormalities, but with a normal karyotype, refused CGH-array compared to 28% of the women with a high risk after combined test principally due to altered biochemistry. Submicroscopic chromosomal abnormalities were detected only in two cases (1.5%). One was an incidental finding with the detection of a microdeletion causative of dystrophinopathy in a female fetus. In the other case, it was a pathogenic 22q11.21 microduplication in a fetus with a NT < 3.5 mm.

Conclusions: CGH-array analysis, performed only after a multidisciplinary counselling, should also be part of the investigation in fetuses with biochemical high risk after a combined test.

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P01.18B

Prevalence of submicroscopic chromosome aberrations in pregnancies without increased risk for structural chromosome aberrations - a systematic review of the literature

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Objectives: To establish the frequency of pathogenic submicroscopic chromosome aberrations in fetuses that are not at increased risk for unbalanced structural chromosome aberrations, a systematic literature search was performed. The aim was to determine whether high resolution testing for submicroscopic aberrations is beneficial in a general pregnant population.

Methods: On 3rd June 2016 Embase and PubMed databases were systematically searched for all relevant articles on prevalence of pathogenic submicroscopic CNVs in fetuses tested due to advanced maternal age or parental anxiety. Relevant full text articles were analyzed and based on the extracted data the prevalence of submicroscopic CNVs was calculated.

Results: Meta analysis was conducted in a pooled cohort of 10,614 fetuses based on the 10 largest studies (N > 300) of a total of 19 relevant studies. In 0.84%, 95%CI [0.55%, 1.30%] of fetuses a submicroscopic pathogenic aberration was detected prenatally. The onset/penetrance of the submicroscopic findings was studied in 10,314 fetuses out of 8 papers that presented aberrant cases with all necessary details. The prevalence of early onset syndromic disorders due to a submicroscopic aberration was calculated to be 1:270, based on 0.37%, 95%CI [0.27%, 0.52%] cases where aberrations were specified.

Conclusions: This systematic review shows that a significant proportion of fetuses in a general pregnant population carry a submicroscopic pathogenic CNV. Based on these figures all women should be informed on their individual risk for all pathogenic chromosome aberrations and not only for common trisomies.

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P01.20D

Chromothriptic events in healthy people: pay attention to "innocent" insertional translocations

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Chromothripsis is characterized by extensive genomic rearrangements consisting in multiple deletions and disordered orientation of the rearranged portions of one or few chromosomes. By whole genome sequencing (WGS) in three unrelated families, we demonstrated that in one parent of each family a balanced chromothripsis was present causing a genomic imbalance in the index case consisting in a deletion and a non-contiguous duplication within 3q22.1-q26.31 in case 1, a simple two-way reciprocal translocation t(6;14) in case 2, and a complex rearrangement involving chromosomes 6, 7 and 15 in case 3. It is noteworthy that a parental chromothripsis at the origin of the proband's imbalance was far from predictable in two of them, in which the proband's rearrangement was at first interpreted as *de novo* in case 1 and consisting in a simple translocation in case 2. In all parents-of-origin a small size fragment of the shattered chromosome was inserted into an additional chromosome. Our findings strongly indicate that (i) both simple and complex unbalanced rearrangements, can in fact be recombinant chromosomes derived by a balanced chromothripsis present in one healthy parent; (ii) WGS investigations in the parent may reveal unexpected genomic complexity that are impossible to foresee by conventional and molecular cytogenetic approaches; (iii) insertional translocations cannot anymore be considered three breakpoints events but rather are the spy of more complex rearrangements. Our partially novel and partially confirmatory data call for WGS as first tier genomic analysis in order to properly evaluate any possible risk for chromosome imbalances at following pregnancies.

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P01.21A

The influence of chromosomal microarray and NIPT on the diagnostic yield in 6811 high risk pregnancies without The influence of chromosomal microarray and NIPT on the diagnostic yield in 6811 high risk pregnancies without ultrasound anomalies

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Introduction: Prenatal diagnostics has been impacted by technological changes in the past decade, which have affected the diagnostic yield. The aim of this study was to evaluate the impact of SNP array and non-invasive prenatal testing (NIPT) on the diagnostic yield and the number of invasive tests in our center.

Material and Methods: The frequency of pathogenic fetal unbalanced chromosome aberrations was studied in 6811 high risk pregnancies without ultrasound anomalies referred for prenatal testing in 2009-2015 due to advanced maternal age, abnormal first trimester screening results (with nuchal translucency < 3.5mm) or recurrence risk for chromosome aberration. Chromosomal SNP microarray analysis replaced karyotyping in in 2012 and since 2014 a choice between NIPT and diagnostic testing with microarray was offered to women with an increased risk for common aneuploidy (as a part of a national TRIDENT study).

Results: The introduction of microarray led to an additional yield of submicroscopic pathogenic chromosome aberrations in 1.9% in fetuses without ultrasound anomalies. The introduction of NIPT led to a decrease of invasive tests, but also of the diagnostic yield.

Conclusions: Since 33% of pathogenic fetal chromosome aberrations were different from the common aneuploidies and triploidy, whole genome analysis should be offered after invasive sampling. Because NIPT (as a second screening) has resulted in a decreased diagnostic yield it should be accompanied by an appropriate pre-test counseling in high risk pregnancies.

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P01.22B

Chromosomal microarray analysis in fetuses with double renal collecting system

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Duplication of the renal collecting system is one of the most common variants of urinary tract anatomy with an estimated incidence of about 1% . This condition is characterized by two pelvicalyceal units draining a single kidney. It can be either complete or partial. The objective of our study was to examine the rate for chromosomal aberrations in isolated prenatal sonographic finding. Data from all chromosomal microarray analyses (CMA) reported to the Ministry of Health between January 2013 and December 2016 were retrospectively obtained from a computerized database. All pregnancies with sonographic diagnosis of isolated duplex renal collecting system and documentation of CMA result were included. Rate of abnormal CMA findings in these cases was compared to that of the general population risk, based on a systematic review encompassing 9272 cases with normal ultrasound, and a local data of 5541 pregnancies undergoing CMA due to maternal request. One pathogenic CMA finding was found amongst 98 pregnancies with double collecting system (1.02%), not significantly different from the risk for abnormal CMA results in the general population. In addition, two variants of unknown significance were demonstrated (2.04%). This is the first report describing the rate of chromosomal anomalies in pregnancies with isolated duplex renal collecting system. It is suggested that routine invasive prenatal testing with CMA analysis in such cases is no more useful than in the general population. Prospective larger studies are needed to guide the optimal management of pregnancies with isolated duplex renal collecting system.

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P01.23C

A component of sperm fibrous sheath has major effect on testis morphology and functionality

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The fibrous sheath is a unique cytoskeletal structure located in the principle piece of the sperm flagellum with more than 13 protein components. AKAP4 is the most abundant protein in the fibrous sheath, which interacts with at least 3 other proteins. The molecular structure and functionality of the fibrous sheath are largely unknown. We collected a clinic sample of sperms featured by dysplasia of fibrous sheath (DFS), leading to a failure of natural conception. The sperm donor is an offspring of consanguineous family, and we identified an inherited homozygous truncating mutation in his genome by whole-exome sequencing. The affected protein is one of the components of fibrous sheath, and this mutation caused a shortened protein which lost part of the original functions. The mice model with this mutation introduced by CRISPR-Cas9 technique showed similar phenotype to the human, with sperms of reduced number and lower motility due to flagellum malfunction. Strikingly, we observed that, unlike AKAP4 knock-out mice model, the knock-out mice model we constructed for the novel gene exerted major effect on testis, manifested by significant size/weight reduction and azoospermia. Microscopic observation of testis slices and in-situ hybridization showed abnormal cross-section of the seminal vesicles and disorganized progression from spermatogonia to spermatid, when comparing the knock-out mice model with the control ones. Therefore, we concluded that this gene is of critical importance to normal spermatogenesis and testis development.

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P01.24D

Use of prenatal exome sequencing in fetuses with ultrasound anomalies

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Introduction: Whole exome sequencing is a diagnostic tool in postnatal settings for individuals with a suspected genetic condition. Recently, its application in prenatal settings has increased and is sporadically used as a diagnostic tool. We present here our experience using this approach in a prenatal setting.

Material and Methods: Exome sequencing was performed in 42 fetal samples carrying different ultrasound anomalies. 30 samples were from evolutive pregnancies and 12 were from legal pregnancy interruptions. In 10 of the samples previous prenatal CGH-array was performed with

negative result. Segregation studies were performed in cases with a candidate variant when possible.

Results: Common reasons for referral were skeletal anomalies, polymalformated fetuses, cerebral anomalies or monogenic disorder suspicion (Noonan syndrome). Pathogenic variants were identified in n = 8 (19%) of samples, being previously described or *de novo* in the index case. In n = 9 (21%) cases, variants of unknown significance were identified, and in two of them inheritance was consistent with expected pattern. In more than half of the cases (52%) we were not able to identify any candidate variant. Diagnostic yield was highest in fetuses with skeletal anomalies, where pathogenic variants were identified in (n = 6) 60% of cases, and in fetuses with clinical suspicion of Noonan syndrome, where a pathogenic variant was found in 75% of the samples. No pathogenic variants were found in polymalformated fetuses or fetuses with cerebral anomalies.

Conclusion: Exome sequencing is a valuable diagnostic tool in fetuses with ultrasound anomalies, especially when skeletal anomalies are present or Noonan syndrome is suspected.

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P01.26B

Challenges and opportunities in variant interpretation in NGS-based expanded carrier screening. First results from an Argentinian cohort

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Introduction: In the past decade, NGS-based technologies have been disruptive in many areas of clinical genetics, mainly related to the diagnosis of known entities. Reproductive medicine has not been excluded from these advances, and expanded carrier screening (ECS) has become increasingly used, both for couples at risk and general population.Here we describe challenges and opportunities of an NGS-based ECS panel for recessive disorders.**Material and Methods:** After variant calling of 120 cases using an in-house developed pipeline for processing data of 483 genes sequenced on a Nextseq 550 platform, variants were classified according to ACMG guidelines and our clinical geneticists' and molecular biologists' criteria.**Results:** 39.2% of the patients were found to be carriers for at least one pathogenic/likely pathogenic variant for 48 different diseases. A pathogenic variant por G6PD (X-linked haemolytic anemia) was found in an apparently healthy man. 91 unique variants of uncertain significance (39 classified as "Conflict of interpretation" in ClinVar) were found in a homozygous/hemizygous state, which when present in genes related to recessive full-penetrance and early-onset disease were deemed likely benign.**Conclusions:** NGS-based ECS represents an unique opportunity to identify couples at risk of having children with recessive conditions but also to calculate variant frequencies from countries underrepresented in global consortiums, diagnose individuals with low-penetrance disorders and classify homozygous/hemizygous variants of uncertain significance as benign. ACMG guidelines are mainly focused on diagnosis of affected individuals, but variant classification for ECS must be based on data not related to the phenotype.

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P01.27C

NGS expanded carrier screening in the Netherlands: initial implementation results

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Introduction: Expanded carrier screening (ECS) has broadened in recent years from high risk population-targeted testing to general public screening. and the main challenge now is choosing the most applicable test design for the intended population. Here we describe the ECS test developed at our department of Genetics and our initial results.

Materials and Methods: Based on focus group discussions, we designed and implemented a couple-based ECS multi-gene test for 70 rare, early onset and serious recessive Mendelian conditions using NGS technologies. Concentrating on couple-based screening, emphasis was on the combined risk for having affected children. The *a priori* risk of being a carrier couple is approximately 1 in 150 and increases for those referred for medical reasons (e.g. consanguinity). Only results with high predictive value regarding affected offspring were reported in the combined result.

Results: A total of 169 couples were tested, 52 potential high-risk couples and 117 general public couples as part of an population-based implementation study. Five couples, referred for diagnostic reasons, shared carriership of one of the diseases tested. All remaining couples tested normal. Reporting times averaged at 38 days, and in some cases even within 2 weeks.

Conclusions: Our combined approach for ECS testing allows for a fast, simplified procedure to report a combined risk to couples, forestalling the burden of individual findings. Broader implementation (e.g. general public via their GP) seems warranted, and is supported by these first results. Future international discussions will guide further development of such important screening tests.

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P01.28D

Molecular autopsy is an important tool in the diagnosis of lethal fetal disorders and structural anomalies

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Introduction: Genomic sequencing is emerging as an important tool in the diagnosis of lethal fetal disorders and structural malformations. We sought to determine the clinical utility of genomic sequencing as an adjunct to standard antenatal imaging and fetal autopsy, as well as the impact of molecular diagnosis on clinical care.

Materials and Methods: We performed a retrospective review of perinatal cases referred to the Monash Genetics Unit between 2015 and 2017 in the setting of structural malformations or fetal-death-in-utero. 19 fetuses were identified in whom genomic testing had been performed following a normal microarray and findings on antenatal imaging and autopsy suggestive of an underlying monogenic disorder. Testing comprised either a targeted panel or whole exome sequencing in a clinically accredited laboratory.

Results: A diagnosis was established in 9/19 cases (47%) on the basis of likely pathogenic or pathogenic variants detected in genes with a concordant Mendelian phenotype: *RIT1, RAF1, L1CAM, AGRN, MTM1, CHRNB1, CEP290, COL1A1*, and *PKHD1*. Variants of unknown significance were detected in 2/19. A significant impact on clinical management was noted in the families who received a molecular diagnosis: 2/9 families were informed that a causative variant was *de novo*, restoring reproductive confidence; 3/9 patients were counselled regarding increased recurrence risk and proceeded to prenatal diagnosis in subsequent pregnancies, and 3/9 couples were referred to an IVF service for preimplantation genetic diagnosis.

Conclusion: Genomic sequencing enhances the diagnostic yield of standard fetal imaging and autopsy and improves patient care.

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P01.29A

NGS studies in structurally abnormal fetuses with a normal chromosomal microarray analysis. Clinical experience

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Objective: The elective genetic testing for structurally abnormal fetuses is chromosomal microarray analysis (CMA). We investigated the value of next generation sequencing (NGS) studies in fetuses with selected structural anomalies and normal CMA.

Methods: During a 30-month period, NGS studies were performed on fetal DNA extracted from amniocytes or chorionic villi in 25 structurally abnormal fetuses with a normal CMA. NGS studies included a single gene analysis (n = 6), gene panel (n = 14), or clinical exome sequencing (n = 5). Single gene analysis was performed in suspected syndromes caused by a single candidate gene (thanatophoric dysplasia, CHARGE, Mowat-Wilson...) and when a panel or exome was not available in our center (Noonan, lissencephaly). A gene panel was used when for a specific fetal syndrome or sign have multiple candidate genes (large echogenic kidneys, Noonan syndrome, craneosynostosis⋯). Finally, clinical exome sequencing was performed in recurrent or multisystem anomalies with no specific syndrome suspicion.

Results: In 40% (10/25) of the cases, NGS provided definitive diagnoses. It was provided in 1/6 (17%) of single gene analyses (thanatophoric dysplasia), in 8/14 (57%) of gene panels (4 by CAKUT panel, 2 by Noonan, one by a craniosynostosis and one by skeletal dysplasia panels), and in 2/5 (40%) of clinical exomes (primary microcephaly and Bohring-Opitz syndrome).

Conclusion: NGS provides a definitive diagnosis in 40% of fetuses with selected structural anomalies and normal CMA results. The most frequent diagnoses were specific skeletal dysplasias, Noonan syndrome in persistent nuchal fold +/- hydrops, and autosomal recessive renal diseases in large echogenic kidneys.

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P01.30B

Fetal exome sequencing: yield and limitations in a single tertiary center

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Objective: To explore the indications and diagnostic outcomes of fetal exomes in a single referral center.

Methods: 77 unrelated fetal samples underwent exome sequencing between 2012-2017. Indications, turnaround time, diagnostic rates, and pregnancy outcomes were analyzed.

Results: The most common indication for fetal exome sequencing was multiple malformations (21/77, 27%), followed by isolated brain malformations (15/77, 19%). Twelve fetuses (15%) were referred for isolated increased nuchal translucency (IINT). Exome analysis was diagnostic for 16 fetuses (21%); when sub-classified to fetal malformations vs. IINT it became clear that exome analysis did not reveal any known or probable pathogenic variants in IINT whereas among the remaining fetuses, a molecular diagnosis was reached in 16/65 (25%). Proband-only cases received a diagnosis more often than trio exomes.

Conclusion: Exome sequencing has the potential to provide molecular diagnoses in cases where conventional prenatal cytogenetic testing is negative. A referral bias of consanguineous cases could account for the high diagnostic rate for proband-only sequencing. Syndrome-specific prognostic information enables parents to make informed decisions, whereas challenges include time limitations and variant interpretation in the setting of non-specific fetal findings. As we report only established disease-gene associations, further segregation and functional studies in a research setting are expected to significantly increase the diagnostic yield.

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P01.31C

Low fetal fraction and digynic triploidy: products-of-conception testing supports Fetal-Fraction-Based Risk model for non-invasive prenatal testing

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Introduction: Pregnancies receiving a 'no result' on non-invasive prenatal testing (NIPT) due to low fetal fraction ('no result' LFF) are at increased risk for trisomies 18 and 13 (T18, T13) and digynic triploidy (DT). The Fetal-Fraction–Based Risk (FFBR) method has recently been validated to assess SNP-based NIPT 'no result' LFF pregnancies given fetal fraction, maternal weight, and gestational age. This method demonstrated high sensitivity for pregnancies affected by T18, T13, and DT (high risk, ≥1%). The objective of this study was to retrospectively apply the FFBR model to SNP-based NIPT 'no result' LFF cases that later had products-of-conception (POC) testing.

Materials and Methods: Over 30,000 consecutive POC samples submitted January 2014-December 2017 were

reviewed. Pregnancies with both SNP-based NIPT 'no result' LFF and SNP-based POC results, which included parent of origin, were selected for application of the FFBR model.

Results: A total of 338 POC cases also had NIPT performed. Of these cases, 48 (14.2%) had 'no result' LFF (expected given its association with pregnancy loss). The prevalence of chromosome abnormalities in the 'no result' LFF cases was 66.7% (32/48); DT was the most common (43.8% [14/32]). All DT cases (100%) received a high FFBR score.

Conclusion: DT was a significant source of chromosome abnormalities among pregnancy losses that previously had a SNP-based NIPT 'no result' LFF. Retrospective application of FFBR demonstrated that these cases could have been identified as at-risk at time of SNP-based NIPT, allowing for a more informed genetic counseling and prenatal management.

S. Krinshpun: A. Employment (full or part-time); Significant; Natera, Inc. **W. DiNonno:** A. Employment (full or part-time); Significant; Natera, Inc. **T. McKanna:** A. Employment (full or part-time); Significant; Natera, Inc. **A. Ryan:** A. Employment (full or part-time); Significant; Natera, Inc. **K. Martin:** A. Employment (full or part-time); Significant; Natera, Inc.

P01.33A

Age-dependent gonosomal mosaicism in fertile women

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Introduction: One of the most significant features of the human genome is high variability. Genomic variations occur during ontogenesis in various tissues and organs leading to the tissue-specific mosaicism. However, phenomenon and mechanisms of a low-level gonosomal mosaicism in women of reproductive age have not been properly described and clarified.

Materials and Methods: preparations from buccal smear (34) and peripheral venous blood (32) each woman had at least one healthy child. Three groups were formed that included woman of different ages: 20-29 years (1), 30-39 years (2) and 40-49 years (3). FISH-analysis with centromeric probes on chromosomes X and 18 in accordance with the standard protocol.

Results: The study found that in the blood of healthy women there is a physiological low-level mosaicism with a clear trend in increased proportion of abnormal cells associated with the increasing age to 1.83%, 2.23% and 5.88% for groups 1, 2 and 3, respectively (P= 0.0042). The buccal smear also exhibited physiological pattern of a low-level mosaicism, however, the level of mosaicism was statistically insignificant in different age groups and, on average, was 4.01% (P = 0.530). It is shown that mosaicism in buccal smear is represented by two cell lines: one is with disomy and another one includes monosomy on chromosome X.

Conclusion: The obtained data can be a reference for the evaluation of low-level mosaicism in fertile women.

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P01.34B

Exome sequencing reveals novel *IGSF10* variation in patients with hypergonadotropic hypogonadism

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Introduction: Hypergonadotropic hypogonadism (HH) is characterized by hypogonadism due to an impaired response of the gonads to gonadotropins (Gn) and a secondary lack of sex steroid production and elevated Gn levels. HH can be caused by environmental factors and congenital disorders that affect ovarian development and function, as well as syndromic and non-syndromic single gene disorders. However, in most cases of gonadal dysfunction the molecular etiology remains an enigma.

Subjects and Methods: To identify novel molecular causes in HH we applied whole exome sequencing (WES) to 33 affected female individuals from 30 unrelated families including 22 with parental consanguinity.

Results: WES revealed likely pathogenic variants in known HH-associated genes in 7/30 families (23%) including *AR, NOBOX, MCM8, PADI6, PSMC3IP*, and *TG*. In seven unrelated families, we identified likely pathogenic variants in candidate disease genes. In three of

these families, each with one apparently sporadic case, biallelic variation in *IGSF10* was found. Two of the three families had reported parental consanguinity. *IGSF10* encodes a 2623-amino-acid member of the immunoglobulin superfamily that is likely involved in controlling early migration of neurons expressing gonadotropin-releasing hormone. All three missense *IGSF10* variants affect conserved amino acid residues, were predicted to be deleterious by several SNV scoring algorithms, and are present in genomic databases with a frequency $<2x10^{-5}$.

Conclusions: A WES approach enabled the identification of novel *IGSF10* mutations in females with HH, thus expanding the spectrum of genes involved in impaired ovarian response to Gn.

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P01.35C

A case of Down syndrome with isodicentric chromosome 21 misdiagnosed by noninvasive prenatal testing (NIPT)

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Isodicentric chromosome 21 is an extremely rare chromosomal aberration. Only several cases have been reported worldwide. The phenotype of patients with 46,idic(21)(q22.3) karyotype is generally concordant with patients who have simple trisomy 21.

We report a girl born in 38-th week from first pregnancy; prenatal screening (USG and a double test) performed in 13-th week of gestation predicted a 6-fold increased risk of Down syndrome. Noninvasive prenatal testing using cffDNA from mother's blood did not show increased risk of trisomy 21 in fetus. Invasive prenatal testing was not performed. After birth, phenotypic features of trisomy 21 were observed in the child.

Cytogenetic testing performed from the periferal blood revealed an unbalanced karyotype 46,XX,idic(21)(q22.3). The result were confirmed using **Methods:** FISH, and MLPA. Microarray additionally revealed a terminal microdeletion sized 11.2 kbp on chromosome 21. Both parents were tested and confirmed negative for any chromosomal aberration from blood and fibroblasts and they have been informed about phenomena of germinal mosaicism in gonadal DNA.

In this case, NIFTY test could not identify the abnormality. Further studies are needed to assess sensitivity of NIPT in the cases of Down syndrome, not caused by simple trisomy 21.

We conclude that invasive prenatal diagnosis should be proposed in all pregnancies with increased trisomy risk, even if NIPT results are negative. High resolution microarray testing can be helpful in identification of microdeletions in patients with idic 21 and could delineate genotype -phenotype correlations.

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P01.36D

De novo mutations in idiopathic male infertility

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Introduction: Infertility affects about 5% of adult human males and despite efforts in understanding genetic basis of male infertility, a large number of cases still remain to be explained. Previous methods of detecting disease-related genes included large family studies. In disorders, such as infertility, natural selection prevents transmission of mutations, and therefore many genes whose mutations cause infertility are not yet known.

Materials and Methods: In order to investigate potential roles of de novo mutations in male infertility we performed trio whole exome re-sequencing in 13 infertile males and their parents. All infertile males were diagnosed with idiopathic azoospermia. For all subjects library preparation was performed with Nextera Coding Exome Capture Kit (Illumina, San Diego, CA), with subsequent sequencing on Illumina HiSeq 2500 platform.

Results: We identified de novo mutations in three infertile males. Among genes with de novo mutations, two (NEURL4, BRD2) were previously implicated in reproduction in animal models. Previous studies have shown that NEURL4 contributes to germ cell formation in

Drosophila, while the BRD2 is essential for chromatin remodeling during spermatogenesis in mice. The third gene with de novo mutation (SEMA5A) has not yet been implicated in reproduction, but it shows expression in testis.

Conclusions: We identified potentially new genes and mechanisms involved in the pathogenesis of male infertility.

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P01.37A

Interphase Chromosome Profling (ICP) as a rapid, sensitive and cost-effective diagnostic tool for Prenatal and Postnatal settings

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Karyotyping has an important role in the genetic work-up of POC specimens, since approximately one half of miscarriages are due to chromosomal imbalances. The three primary methods used to obtain karyotype are 1) Classical cytogenetics 2) Targeted FISH and 3) aCGH. Each of these methods has its advantages and disadvantages. Additionally, the TAT is significantly long. The targeted FISH, only covers 5-7 chromosomes and therefore provides incomplete information. It cannot detect any structural abnormalities. Prenatal diagnosis by karyotype determination is done mostly to provide assurance, since majority of the pregnancies would have a normal karyotype. Therefore, fast and accurate information is highly critical for management of the pregnancy. However, the same limitations mentioned for POC also apply to prenatal diagnosis. To overcome these challenges, we recently validated and adapted a novel cytogenetic technology 'Interphase Chromosome Profiling (ICP) (InteGen LLC, USA) to assess the molecular karyotype of 200 miscarriage material and 80 amniotic fluid samples using interphase nuclei. For POC and AF samples, using ICP probes, all numerical, most balanced and unbalanced structural aberrations, and all Robertsonian translocations can be detected. Using ICP, we obtained results from all (100%) samples and the TAT was significantly reduced to less than 1 day. However, with a proper workflow, results can be delivered in less than 2 hours.

S.K. Bhattacharya: A. Employment (full or part-time); Significant; Dr. Lal PathLabs Ltd. **V. Lal:** A. Employment (full or part-time); Significant; Dr. Lal PathLabs Ltd..

P01.38B

The impact of chromosomal microarray analysis on resolving intra-utrine fetal death cases in Israeli population

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Background: 30-50% of clinically recognized pregnancies end in intra-uterine fetal death (IUFD), 20% within the second and the third trimester, 25-60% without determined cause. Genetic work-up may lower the uncertainty, acknowledge the recurrence risks and enable wiser management of future pregnancies. Microarray technology (CMA) plays a major role in identifying genetic etiology of prenatal and postnatal pathologies, raising detection rate compared with karyotyping. An advantage of CMA in IUFD workup, is usage of directly extracted DNA without a culture, therefore without viability issues. The CMA chip includes copy number and singe nucleotide polymorphism probes, enabling identification of small copy number changes, mosaicism and homozygous regions throughout the genome. We charachterized CMA findings in IUFD cases in the Israeli population to find: - the characteristics and frequency of the chromosomal aberrations in IUFD compared to livebirths. - Do maternal and fetal determinants have impact on chromosomal aberration prevalence in IUFD cases? - Can finding of higher percentage of homozygous regions in IUFD explain the etiology?

Methods: We performed CMA on placental tissue of IUFD, gathered information regarding mothers and fetuses charachteristics, and compared with information of women who underwent prenatal diagnosis during the same period.

Results: CMA finding was an independent predictor with higher prevalence in IUFD than in live-born pregnancies. The chance of finding a pathogenic aberration in in IUFD with congenital anomalies was higher than other women. Homozygosity analysis had no advantage over CNV analysis.

Conclusions: CMA resolves more IUFD cases and therefore should be implicated in such cases.

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P01.39C

Extended genetic analyses in infertile men with non-obstructive azoospermia

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Male infertility is a clinically and genetically highly heterogeneous disease, mostly caused by spermatogenetic failure. The most severe form is non-obstructive azoospermia (NOA). In the majority of NOA cases, a genetic origin is suspected but current genetic testing, comprising cytogenetic analysis and AZF deletion screening, only discovers the cause in about 17%.

Recently, we expanded our analyses of NOA patients that attended the Centre of Reproductive Medicine and Andrology (CeRA). First, the routine chromosomal and AZF analyses were performed. In a second step, sequence analysis of three genes was carried out.

Chromosomal analyses were performed in 399 patients of whom 60 (15.0%) were identified with numerical (47,XXY; 47,XYY) or structural aberrations (46,XX; aberrant Y chromosomes; translocations; inversions). AZF deletions were found in 1.8% (6 of 335).

The coding sequence of TEX11, NR5A1 and DMRT1 was analysed in 123 patients. Potentially pathogenic variants were identified in 6 patients (4.9%). One mutation in TEX11 (c.450C>T) and one in NR5A1 (c.712G>A) were already published, whereas novel mutations were detected in NR5A1 (1x c.1079C>T) and in DMRT1 (2x c.308A>G, 1x c.436C>G).

In conclusion, the basic genetic analyses in men with NOA by conventional cytogenetic analysis and AZF screening revealed the expected number of aberrations. Through sequencing of three genes, which have been confirmed as responsible for spermatogenetic failure, an additional 5% of men carrying possibly pathogenic variants were identified.

This work was carried out within the frame of the DFG Clinical Research Unit 'Male Germ Cells: from Genes to Function' (CRU 326).

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P01.40D
Profile of copy number variants in Estonian men with impaired spermatogenesis

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Introduction: Infertility affects 5-7% of men. The current pipeline at the andrology clinic is able to assign a definite cause for 40% of patients, including genetic errors in 8% of cases, whereas 60% of them remain idiopathic (Punab M *et al.* 2017 Hum Reprod). Considering the complexity of spermatogenesis, it is likely that a substantial proportion of this uncharacterised aetiology may be explained by unknown genetic factors. Currently, the role of genomic copy number variants (CNVs) in male infertility is not well defined. We aimed to characterise genome-wide profile of CNVs among Estonian men with idiopathic infertility.

Material and Methods: All patients (n = 211) and controls (n = 100) were recruited and clinically characterized at the Andrology Centre, Tartu University Hospital. Cases comprised of idiopathic non-obstructive azoospermia or oligozoospermia patients. Controls represented partners of pregnant women. CNV calling utilized genome-wide SNP data (HumanOmniExpress-24 BeadChip) and was performed using alternative CNV prediction algorithms in parallel. CNVs were validated with TaqMan (specific loci) or aCGH (microdeletions/duplications).

Results: Infertility patients and fertile men did not differ in their overall CNV load. However, an enrichment of asymptomatic carriers of known microdeletions and microduplications was observed among patients. Additionally, a novel recurrent CNV overlapping an uncharacterized testis-specific gene, was detected solely in seven infertility cases. Replication analysis for its association with male infertility is ongoing in a larger Estonian cohort.

Conclusions: Diagnostic yield for the patients with impaired spermatogenesis may be increased via introducing profiling of genome-wide genomic rearrangements into clinical routine.

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P01.41A
Two novel CEP290 pathological variants prenatally identified by targeted next-generation sequencing using a custom Meckel-Gruber gene panel

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Meckel-Gruber syndrome (MKS) is a lethal autosomal recessive disorder characterized by a classic ultrasound triad of occipital encephalocele, polycystic kidneys and postaxial polydactyly. anomalies of the central nervous system, dysplasias and malformations. The mortality is 100%.. In this case report, a prenatal sample from a fetus with MKS clinical features was screened for 21 genes using a targeted next-generation sequencing panel using a Ion PGM System (Thermo Fisher Scientific).

Two novel pathological variants, both resulting in stop codons, p.Ser1198* (c.3593C>A) and p.Ser1648* (c.4943C>G), were found in the CEP290 gene which codes for a centrosomal protein of 290 kDa involved in early and late steps of cilia formation. Sanger sequencing confirmed the carrier status of the parents.

Our results show that our Meckel-Gruber targeted next-generation sequencing 21-gene panel is an effective tool for the identification of pathological variants involved in this syndrome and confirms the possibility of obtaining a faster and accurate prenatal genetic diagnosis.

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P01.43C
Comparison of microRNA profiles in infants born with and without assisted reproduction techniques

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The incidence of congenital anomalies in ART babies is higher than that of babies born with spontaneous pregnancy. The cause of this situation is unknown. Three mechanisms are known to cause congenital anomalies in ART pregnancies: point mutation, chromosomal disorders, epigenetic abnormalities. An important factor in epigenetics is microRNA. Studies have shown microRNAs are associated with fertility and development.The aim of this study to demonstrate whether infertility-related miRNAs are different in children born with spontaneous pregnancy compared to those born with ART. The other aim of the study to show whether miRNAs are associated with anomalies and dysmorphic findings in patients. A total of 38 term newborns included the study. In Akdeniz University Hospital, a baby born with 21 ART and 17 spontaneous pregnancies within one year was included in the study. Plasma samples were taken from newborns. All newborn's physical examinations were performed and enrolled. The total microRNA isolated from plasma samples was reverse transcribed into the cDNA. Quantitative real time -PCR was performed with specific primers for miR-16 reference gene and miR-17, 21, 23, 92, 141, 145, 191, 483 target genes. REST software was used for the normalization of relative expression values. The plasma levels of the target miRNA molecules were showed comparable difference between control and ART groups. All three target miRNA molecule were displayed significantly higher levels of expression in ART babies than controls. In our preliminary results signed that infertility-causing miRNAs in parents might be cause of congenital anomalies in newborns. Project code:TTU-2016-1709

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P01.44D
Feasibility and concerns of preimplantation genetic diagnosis for mitochondrial DNA disorders

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Preimplantation genetic diagnosis (PGD) is an alternative procedure to prenatal diagnosis for couples at-risk to have children affected with a severe genetic disease, such as a mitochondrial DNA (mtDNA) disorder. PGD relies on the genetic analysis of one or a few cells sampled from in-vitro fertilized embryos, between day 3 and day 5 of development. In the case of mtDNA disorders, quantification of the mutant load on these cells is performed in order to assess the risk for the embryo to develop a severe mitochondrial disease, either in utero or in childhood. Our 15-year experience supports the reliability of such procedure. Overall 15 heteroplasmic patients were included in our PGD program. A total of 26 cycles were started, 25 oocytes retrievals and 16 embryo transfers were performed, resulting in 3 pregnancies and birth of 3 children. The mutant load assessed on a single blastomere sampled from 94 embryos was very close to the mutant load of the remaining cell/embryo, for all mutations tested: m.8344A>G, m.3243A>G, m.8993T>G, m.8993T>C, m.9185T>C,

m.10197G>A. Most of the transferred embryos (17/25) were heteroplasmic, and 2/3 neonates carried the maternal mtDNA mutation, questioning the long-term prognosis of these patients. PGD remains a cumbersome procedure with a low success rate, which cannot be applied to homoplasmic or critically homoplasmic patients. There is therefore a strong need to develop alternative procedures such as nuclear transfer.

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Detecting confined placental and fetal mosaicism using cell-free DNA sequencing on maternal plasma

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Introduction: Mosaicism is characterized by a normal and an abnormal cell-line. Test results on cell-free DNA (cfDNA) in maternal plasma can be compromised as the fraction of abnormal cells may be too low for detection. We wanted to explore the detection rate of both confined placental and fetal mosaicism using cfDNA sequencing on maternal plasma.

Methods and Material: We retrieved data on invasive samples from mosaic pregnancies obtained from 2014 to 2017. On maternal plasma, we retrospectively performed cfDNA testing by genome-wide massive parallel sequencing and VeriSeq-NIPT analysis software.

Results: CfDNA detected placental mosaicism in 59% ($n = 16$). The false negative rate of placental mosaicism using cfDNA testing was 41% ($n = 11$). The mean level of mosaicism in the invasive samples was 72.0% in the detected cases and 20% in the false negative cases ($p < 0.05$). Fetal mosaicism, confirmed by amniocentesis, was detected by cfDNA sequencing in 63% (5/8 cases). A mosaic trisomy 21 case which was confirmed both by CVS (84% T21 cells) and AC was missed by cfDNA.

Conclusion: CfDNA sequencing is capable of detecting placental mosaicism in 59% of the cases. It seems that the level of mosaicism in the invasive samples predicts whether or not cfDNA testing is able to detect the abnormal cell-line; however a high-level mosaicism of trisomy 21 was missed. In the future, we need to learn more about placental mosaicism in general and in particular the comparability between the detection rates of the new non-invasive methods.

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A homozygous donor splice-site mutation in the meiotic gene *MSH4* causes primary ovarian insufficiency

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Premature ovarian insufficiency (POI) is a pathology affecting women under 40 years of age characterized by an early cessation of menses and high FSH levels. Despite recent progresses in molecular diagnosis, the etiology of POI remains idiopathic in most cases. Whole-exome sequencing of members of a Colombian family affected by POI allowed us to identify a novel homozygous donor splice-site mutation in the meiotic gene *MSH4* (*MutS Homolog 4*). The variant followed a strict mendelian segregation within the family and was absent in a cohort of 135 women over 50 years of age without history of infertility, from the same geographical region as the affected family. Exon trapping experiments showed that the splice-site mutation induced skipping of exon 17. At the protein level, the mutation p.Ile743_Lys785del is predicted to lead to the ablation of the highly conserved Walker B motif of the ATP-binding domain, thus inactivating MSH4. Our study describes the first *MSH4* mutation associated with POI and increases the number of meiotic/DNA mismatch repair genes formally implicated as being responsible for this condition.

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Clinical application of paired-end MPSS for cfDNA screening of common aneuploidies

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Paired-end MPSS allows digital counting of plasma cfDNA while also measuring fragments length. cfDNA size differences can be used to determine fetal fraction (FF) and to improve sensitivity by additionally applying counting statistics on short (fetal) fragments.

NeoBona is the first test using such approach, we evaluated its performance by screening a large cohort of consecutive average risk gestations.

Prospective study of 19151 pregnancies (575 twins) screened for common trisomies, including XY aneuploidies in 57% of cases.

NeoBona test was used to determine the likelihood of aneuploidy (Tscore) based on FF, counting statistics and cfDNA size distribution where cut-offs were applied to classify normal and aneuploid cases.

Test results were provided in 99.2% gestations, 288 T21, 63 T18 and 27 T13, in 23 cases detected with FF between 0.8 and 3%. Invasive procedures were performed in 99% risk pregnancies, 5 false positives were observed for T21, 2 T18 and 3 for T13 (FPR 0.03%, 0.01% and 0.02%); 1 T21 was missed (DR 99.7%). XY aneuploidies were reported in 39 cases, follow-up available for 14 with 4 FP results (FPR 0.13%). Vanishing twins of discrepant sex were suspected in 5 cases and 4 maternal X aneuploidies were identified.

Paired-end MPSS and the bioinformatics approach of NeoBona allowed detecting aneuploidies even at fetal fractions below 1% while reducing FPR. Removing the need of a lower FF limit allowed cfDNA analysis to be successful on a high proportion of clinical cases extending the benefits of cfDNA screening to a larger population of pregnancies.

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Map of maternal copy number variation highlighted by NIPT analysis

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Since July 2017 reimbursement of NIPT is entirely covered during pregnancy for all pregnant women in Belgium. In our institute we processed more than 12000 samples over a 6 month period. Our in house NIPT workflow allows the identification of trisomies involving chromosome 13, 18 and 21 but also trisomies affecting other autosomes as well as sex chromosomes aneuploidies. Intrachromosomal rearrangements are also investigated using a 1 Mb sliding window approach. This allowed us to identify fetal rearrangements in a number of cases but also maternal rearrangements with an unpreceded power. Indeed given the prevalence of maternal cfDNA in purified cfDNA prepared from the blood of pregnant mothers, some rather small rearrangements can be pin-pointed quite reliably. Interestingly, some unusually large maternal rearrangements (>1 Mb) have also been observed. Maternal CNVs can usually be distinguished from fetal ones by the level of significance of the intrachromosomal Z-score. Some of these CNVs were futher confirmed by CGH arrays using maternal constitutional DNA. The majority of these CNVs are duplications (68 duplications and 43 deletions) some of which overlap syndromic genes. Some correspond to large known CNVs. However, some large CNVs ranging from 1 to 5 Mb seem to be rare familial deletions or duplications probably not associated with any pathogenic phenotype. Indirect screening of the whole maternal population using NIPT offers a unique opportunity to identify large probably benign CNVs. A map of these rare familial CNVs characterized by CGH will presented.

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External assessment of the quality of cell free fetal DNA non-invasive prenatal testing for aneuploidies

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Introduction: To deliver a high standard of laboratory testing, external quality assessment (EQA) is required to provide important information for clinicians, laboratories and patients, demonstrating that accurate testing is being performed and reported. Providing an EQA for cell free fetal DNA (cffDNA) testing is challenging as sample acquisition (availability and scalability) is a limiting factor. The delivery and results of a large international pilot EQA for laboratory NIPT for aneuploidy using maternal plasma samples is described.

Materials and Methods: Eighty-six maternal plasma samples from pregnancies with known outcomes (low/high-risk for common aneuploidy) were obtained from the RAPID sample bank. Three EQA providers (CEQAS, EMQN, and UKNEQAS for Molecular Genetics) delivered the pilot assessing NIPT and reporting The submitted reports were assessed and feedback provided for genotyping, interpretation and clerical accuracy.

Results: Ninety-five laboratories from 30 countries participated. The use of maternal plasma allowed any testing methods to be applied. Two critical errors were reported; one false positive and an incorrect high-risk trisomy 18 result. Reports lacked details of methods, limitations, and many formats made it difficult to identify key clinical recommendations.

Conclusions: Growing international demand for participation demonstrates the clinical need for an independent evaluation of NIPT practice. This pilot EQA has demonstrated that the use of real maternal samples distributed at ambient temperature has enabled global participation with very low sample failure rate (2%). The genotyping accuracy was good but review of the large number of reports submitted highlighted the need for further standardisation and guidance on NIPT reporting.

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Validation of novel bioinformatic algorithm SCAR for fetal sex determination in twin pregnancies

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Introduction: Currently used approaches for determination of fetal sex in noninvasive prenatal testing (NIPT) have shown limitations in correct prediction of fetal sex in cases of twin pregnancies. According to recent information only SNP based tests of NIPT category are able to determine fetal sex for each of the twin.

Aim: To test the feasibility and to validate the new bioinformatic algorithm called SCAR to predict sex of both fetuses in twin pregnancies with utilisation of whole genome coverage genomic scan of circulating DNA from pregnant plasma.

Materials and Methods: Low coverage whole genome sequencing analysis was performed on MiSeq and NextSeq platforms on circulating DNA of 76 pregnant women with twins according to previously published protocol. For each of the fetuses sex determination algorithm SCAR predicted the most probable combination of twin sexes: girl-girl; girl-boy or boy-boy according to fetal fraction counted from fragment lengths and reads mapped on Y chromosome. All predictions were verified after delivery.

Results: Among 76 twin pregnancies, 70 were identified correctly and 6 cases were found as uninformative. All of 6 samples fell to the group with fetal fraction lower than 10%.

Conclusion: Novel algorithm SCAR predicted correctly 92% cases however fetal fraction under 10% critically affected reliable sex determination in boy-girl and boy-boy pregnancies.

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P01.52D

Chromosomal microarray coupled to genome-wide non-invasive prenatal testing (NIPT) to minimize the number of unnecessary invasive procedures

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Prospective clinical results on genome-wide NIPT are still few and there is little follow up and little data available on test accuracy. We received 1921 samples including 108 twin pregnancies for NIPT. 93.6% of the cases were analyzed genome-wide. Additionally, in order to assess test limitations, we performed NIPT retrospectively in 90 cases with a variety of segmental aberrations. In the prospective cohort, 176 samples showed chromosomal abnormalities, 144 of small size. In the latter, we performed chromosomal microarray analysis on maternal DNA obtained from the NIPT tube and suggested invasive testing only for

aberrations not of maternal origin. We found maternal CNVs in 7.8% of the total cases which were surprisingly large in several instances. We detected and confirmed pathologic chromosomal abnormalities in 32 samples, six of which would not have been detected if NIPT had been restricted to common trisomies. The positive predictive value for the common trisomies was 100% and a retrospective questionnaire for quality control showed no evidence for a false negative result. The positive predictive value for non-maternal segmental anomalies was 50%. Thus the number of ''unnecessary'' invasive procedures provoked by genome wide NIPT was as low as 0.3%. We correctly detected all chromosomal aberrations bigger than 6.3 Mb in size in the retrospective cohort. Altogether, we demonstrate that genome wide NIPT does not lead to a significant loss of specificity, if in cases with segmental abnormalities, maternal chromosomal microarray testing is performed prior to invasive testing.

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Non-invasive prenatal testing of microdeletion syndromes

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Introduction: The discovery of cffDNA in maternal plasma has greatly facilitated the development of NIPT of fetal aneuploidies. However, sub-chromosomal copy number change detection still remains a challenge. Towards this goal, we employed a proprietary hybrid capture-based technology and novel bioinformatics pipeline for the detection of microdeletion syndromes. By leveraging the inherent high enrichment uniformity and high read depth of this in-solution hybridization NIPT method we achieved accurate non-invasive detection of fetal microdeletion syndromes. The assay combines multiple depth of coverage-based and fragment size-based ploidy detection engines to detect 1p36, DiGeorge, Wolf-Hirschhorn, and Smith-Magenis microdeletion syndromes with high sensitivity and specificity.

Materials and Methods: cfDNA was extracted from 752 unaffected first trimester pregnancy plasma samples and 29 affected prenatal and synthetic samples. Enrichment probes were designed to span the syndromes' critical regions avoiding low copy repeats and repetitive elements. All samples were enriched using hybrid capture technology as previously described. Enriched sequencing libraries were analyzed using a proprietary statistical analysis pipeline developed to test for deletions in each of the syndromes.

Results: The assay was able to correctly classify all abnormal and normal samples resulting in 100% specificity and specificity.

Conclusions: Using a proprietary target capture enrichment technology and novel multi-engine copy number detection pipeline we accurately detected all normal and abnormal samples. This novel microdeletion NIPT method overcomes the limitations of other methodologies and increases the number of diseases that can be reliably detected by NIPT, thus offering more choices to couples towards an informed management of their pregnancy.

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P01.54B

Development of a novel noninvasive prenatal test (NIPT) of fetal aneuploidies, microdeletions and 50 single gene diseases

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Introduction: We hereby present a novel NIPT of major aneuploidies, microdeletions and 50 monogenic diseases with moderate and severe phenotypes, including Hematological, Kidney, Opthalmological, Neurological, Inherited Metabolic Diseases, such as Thalassaemia, Cystic Fibrosis, Phenylketonuria and Tay-Sachs.

Methods: cfDNA was obtained from 300 pregnancies referred for NIPT at 10^{th}-15^{th} week of gestation for identification of 651 causative mutations in 50 disease associated genes. A study including another 1000 pregnancies using cfDNA and paternal DNA is ongoing for NIPT of major aneuploidies, microdeletions and 50 monogenic diseases. An enriched sequencing library was prepared using custom TArget Capture Sequences (TACS) as previously described. TACS were designed based on genomic locations of known causative mutations for monogenetic diseases under investigation. Enriched

products were sequenced using NGS and the data was processed using a custom bioinformatics pipeline.

Results: For the initial 300 samples, a high number of causative mutations was identified and a selection of those was confirmed using Sanger sequencing. For the ongoing 1000 samples, causative mutations were identified and the fetal risk for aneuploidies, microdeletions and monogenic disorders was determined.

Conclusions: This is the first time that NIPT is made available for a high number of single gene diseases together with aneuploidies and microdeletions, opening a new chapter in prenatal screening. The cumulative risk for the fetus is estimated to be as high as 1/125. This novel NIPT is expandable to hundreds of single gene diseases and can be taken potentially by all pregnant women as early as the 10^{th} week of gestation

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P01.55C
Non-invasive prenatal testing (NIPT): how to handle secondary findings of maternal chromosomal abnormalities

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NIPT has become a widely implemented screening test for the detection of fetal trisomy 13, 18 and 21. Over 8000 NIPT analyses have been performed thus far at the Center for Medical Genetics Ghent, using shallow whole genome sequencing (sWGS) protocol. Around 0,6% samples showed an increased risk for trisomy 13, 18 or 21. Also in 0,6% analyses, we reported another chromosomal abnormality, including other fetal aneuploidies but also several clinically relevant maternal CNVs. The detection and disclosure of these (secondary) maternal aberrations poses ethical dilemmas. Aberrations such as the unfortunate detection of a (predisposition to) malignancy or other incidental findings are not always straightforward to disclose.

We recently identified a possible malignancy in a 25-year old women. The chromosome profile resembles aberrations previously seen in patients with colon carcinoma (loss of 8p, gain of 8q and 20). During subsequent colonoscopy, a possible precursor adenomatous polyps was removed. Follow-up is ongoing. Also, some well-known (maternal) CNVs have been identified, such as Hereditary Neuropathy with liability to Pressure Palsies (HNPP) deletions (OMIM162500). Furthermore, several deletions and duplications with unknown clinical significance have been detected that affected the chromosomal Z-scores of the NIPT analysis. In five cases thus far, the presence of an extra X-chromosome in the mother was present, these were communicated.

With the plummeting costs of NGS on the one hand and the advent of better bio-informatic tools for analysis on the other hand, guidelines are clearly needed to guide us in this new genetic, healthcare landscape of secondary findings.

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P01.56D
Clinical experience with noninvasive prenatal testing (NIPT) for rare autosomal trisomies

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Objective: Using a whole-genome sequencing NIPT approach, our laboratory began offering screening for rare autosomal trisomies (RATs) in 2017. This study presents our initial clinical experience.

Method: Maternal blood samples from over 10,000 singleton pregnancies were analyzed in the CLIA-certified Illumina Laboratory (Redwood City, CA) by the Verifi™ Plus Prenatal Test. Sequencing data was computationally processed with chromosome-specific quantitative scores determined using sequence coverage and fetal fraction. Classification thresholds for each chromosome were derived to maximize specificity while accounting for differences in prevalence for each RAT.

Results: 43 cases (0.4%) were reported as RAT screen positive. The most common RAT identified was trisomy 22, followed by trisomies 7 and 9. The average maternal age (35.0 years) and gestational age (12.4 weeks) of the screen positive cohort were similar to the whole study cohort. However, some high-risk indications, abnormal ultrasound (1.8x) and history suggestive of increased risk for

aneuploidy (5.5x), were more frequently listed in the screen positive cohort than in the whole study cohort. Clinical outcome was available in 8 cases (18.6%): 2 confirmed positives (1 full trisomy 9; 1 segmental 9p duplication), 2 false positives, 3 miscarriages, and 1 elective termination without confirmatory testing; >99% of pregnancies are ongoing.

Conclusions: Our 0.4% screen-positive frequency is consistent with previous studies, though some differences were noted in the relative RAT prevalence.[1,2] Results obtained through NIPT early in pregnancy can be valuable for clinical management. Ongoing outcome collection will provide more insight into the biological aspects of RATs.

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P01.57A

Outcome of high risk for digynic triploidy results on SNP-based non-invasive prenatal testing

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Introduction: Single nucleotide polymorphism (SNP)-based non-invasive prenatal testing (NIPT) is uniquely able to identify the extra haplotype and parental origin in triploid pregnancies. The objective of this study was to establish a positive predictive value (PPV) for pregnancies suspected to be at high risk for digynic (maternal) triploidy (DT) via SNP-based NIPT. For apparent false positive cases, possible maternally-derived causes were investigated.

Materials and Methods: Retrospective outcome data were collected for SNP-based NIPTs performed between January 1, 2015 and December 31, 2017 and coded as high risk for DT. IRB-approved outcomes comprised: number of fetuses, ultrasound findings, results of cytogenetic testing including parental origin of triploidy, and maternal medical findings.

Results: A total of 39 cases with suspected DT were identified and outcome data were obtained for 30 (77%) cases (see Table). The PPV for confirmed or suspected triploidy was 23.3% (7/30). Maternal neoplasm was found in 26.7% (8/30) cases.

Conclusions: This post hoc analysis of SNP-based NIPT data revealed a PPV of 23.3% for pregnancies determined to be at high risk for DT. An additional and unexpected finding was the similar number of maternal neoplasm cases. While a small cohort, these results suggest that maternal neoplasm should be included in the differential diagnosis of high risk DT results on SNP-based NIPT.

Table. Outcomes from suspected DT pregnancies determined via SNP-based NIPT

Outcomes, n (%)	Cases (N=30)
Normal fetal and maternal outcome	12 (40.0)
Maternal neoplasm[a]	8 (26.7)
Triploidy suspected by ultrasound	4 (13.3)
Confirmed triploidy	3 (10.0)
Complete molar pregnancy	1 (3.3)
Early fetal demise	1 (3.3)
Ongoing early gestation	1 (3.3)

[a] 4 lymphoma, 1 colon cancer, 1 Stage IV cholangiocarcinoma diagnosed a year after delivery, 1 ovarian teratoma, and 1 unspecified.

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P01.58B

Widespread use of Non Invasive Prenatal Testing(NIPT) : experience of a Belgian genetic Center

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Non Invasive Prenatal Testing was developed in our institute in 2014 using an in house whole genome approach. For more than 3 years, this test was open for all pregnant women at their own expense. During this period we tested 7041 maternal blood with a positive screening rate for chromosome 13, 18 and 21 of 0,10%, 0,20% and 1,3% respectively. Since July 2017 NIPT has been reimbursed in Belgium for all pregnant women. This resulted in a sharp increase in activity as in 6 months more than 12000 samples were processed. The rates of trisomy 13, 18 and 21 over this period were 0.05%, 0.06% and 0.4% respectively. Beside

these common trisomies other trisomies involving chromosomes 4, 6, 7, 8, 14, 15, 16, 20 and 22 were identified, most of them (>94%) were not confirmed on invasive samples. In case of trisomy for chromosome 6, 7, 11, 14, 15 and 20, uniparental disomy was evaluated in the normal fetus. Intrachromosome analysis using a 1 Mb sliding window approach, allowed to identify some micro rearrangements affecting the fetus. These ones ranged from a few megabases to several tenth of megabases and were confirmed by CGH on amniocytes. Sex chromosome aneuploidies could be technically identified but a Belgian prenatal consortium (www.BeSHG.be) decided not to report these sex chromosomes aneuploidies. Indeed generalization of NIPT would screen almost the whole population for these sex aneuploidies as well as for susceptibility loci. This raised ethical questions which have to be addressed.

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P01.59C
Making NIPT available to all pregnant women

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Non-Invasive Prenatal Testing (NIPT) is increasing in interest for detection of aneuploidies due to these tests giving a more reliable result than obtained from traditional first trimester screening. NIPT should not only be available to high-risk pregnancies but for all women. With Vanadis NIPT, the aim was to fulfill this criterium, making NIPT available for all women by creating a fully automated method with simple preparation need and minimal hands-on time and thereby reducing both complexity and cost. Vanadis NIPT is a sequencing and PCR free, probe-based technology used to label targets on chromosome 13, 18, 21 and Y, thereby allowing for trisomy screening (13, 18 and 21) as well as sex determination. The assay consists of four enzymatic steps resulting in Rolling Circle Amplification Products (RCPs) for each of these four chromosomes. The RCPs are labeled with four different dyes, one for each chromosome, and deposited onto a nano-pore filter from where the labeled objects are counted by imaging. We will present performance characteristics for the Vanadis NIPT assay, including data for real clinical samples, to show the analytical precision and clinical feasibility to correctly identify trisomy 13, 18 and 21 as well as the sex of the fetus.

Å. Janfalk Carlsson: None.

P01.60D
Non-invasive prenatal diagnosis (NIPD) of single gene disorders by relative haplotype dosage (RHDO): review of 18 months of clinical service

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Introduction: We have developed and implemented a relative haplotype dosage (RHDO)- based method for NIPD of multiple single gene disorders (SGD), including spinal muscular atrophy (SMA), Duchenne and Becker muscular dystrophies (DMD/BMD), cystic fibrosis (CF) and congenital adrenal hyperplasia (CAH). Diagnostic services for SMA and DMD/BMD were launched in September 2016, followed by the launch of a CF service in December 2017.

Materials and Methods: The test involves targeted enrichment of thousands of SNPs across multiple genomic regions and massively parallel sequencing (Illumina MiSeq) of cfDNA followed by RHDO analysis. Maternal, paternal and proband genomic DNA samples are tested alongside cfDNA for haplotype phasing and to measure fetal fraction. Our method can test 2-3 pregnancies on a single MiSeq run, thus centralising testing and decreasing costs. The development of an automated analysis pipeline has increased capacity further. There is no requirement to confirm positive results.

Results: To date, we have performed NIPD for 48 referrals (UK and international) and reported 16 normal, 18 unaffected carrier and 10 affected pregnancies. For 4 cases, a complete result could not be issued due to persistent low fetal fraction, a recombination event or lack of informative SNPs. Of the 48 diagnostic tests, we have so far received postnatal confirmation of 15 results, with no discrepancies.

Conclusions: NIPD by RHDO is a robust assay, which is feasible to provide in a clinical setting for both X-linked and autosomal recessive disorders. The assay could be extended to increase the availability of NIPD for many monogenic disorders.

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P01.62B
Predicting fetoplacental chromosomal mosaicism during non-invasive prenatal testing

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Objective: Non-invasive prenatal detection of trisomies 21, 18 and 13 can be achieved with high accuracy through sequencing of cell-free DNA (cfDNA) found in maternal blood. Using a genome-wide approach, fetal aneuploidies other than the common trisomies can also be detected. Fetoplacental mosaicism is the main cause for false positive/negative NIPT results. We further improved the analytical power of genome-wide cfDNA screening by enabling the detection of fetoplacental mosaicism.

Method: Aneuploidy detection was combined with fetal fraction estimation to enable the detection of placental chromosomal mosaicism. This pipeline was applied to whole genome sequencing data derived from ~20.000 maternal plasma samples. Following an abnormal NIPT, test results were validated by conventional invasive prenatal or postnatal genetic testing.

Results: The new analysis pipeline identified 134, 24 and 7 non-mosaic trisomies 21, 18 and 13 respectively. All for whom follow-up information was available were confirmed upon invasive testing. The incidence of other, rare autosomal trisomies (RATs) was ~0.3%, with trisomy 7 and 16 being the most prevalent. Three of these RATs, predicted as full trisomies in the placenta, were found to be mosaic in the fetus; 25 other RATs were predicted to be mosaic, 8 of which have been confirmed in placental tissue. The new pipeline also correctly predicted twin pregnancies with discordant fetal sex.

Conclusions: This improved analysis pipeline permits the detection of autosomal aneuploidies and pinpoints pregnancies at risk of fetoplacental mosaicism. This knowledge can influence estimation of the risk for miscarriage, aid in genetic counselling and improve prenatal management.

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P01.63C

Pregnancy outcome for fetuses with increased nuchal translucency but normal karyotype and CMA: impact on the counselling and need for further genetic investigations

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Objectives: To investigate the outcome for fetuses with nuchal translucency (NT) ≥3.5 mm but normal karyotype/CMA.

Methods: All patients were referred to our institution for NT≥3.5 mm from 2012 to 2016. We followed prenatally all patients until delivery and pregnancy outcome was recorded. Targeted Resequencing was performed using a panel including 15 RASopathies genes.

Results: We identified 74 fetuses. An adverse perinatal outcome was observed in 27% of cases. US follow-up showed 10 cases with cardiac malformations (major in 6/10); diaphragmatic hernia (1); Dandy Walker Malformation plus skeletal dysplasia (1); pyelectasis (1), and IUGR (1 monochorionic twins). 54% of these cases (40/74) were analyzed using our customized panel of RASopathies genes. Four variants were identified, the aminoacid substitutions Val1432Phe and Arg2452Cys, in the *NF1* gene, and a Thr7Arg and Thr159Pro, in the *LZTR1* gene. Four additional intronic variants were also identified, but no one altered the splice site according to the prediction tools. Thirty additional fetuses with NT ≥ 3.5 mm had been previously analyzed, leading to the identification of two variants, the Gln506Pro in the *PTPN11* gene, and the Glu63Lys in the *KRAS* gene.

Conclusion: Even with normal karyotype/CMA, a NT>99th centile is associated with an adverse pregnancy outcome, as in one third of cases a congenital malformation and/or a miscarriage was observed. More interestingly our study demonstrate a RASopathy gene variant in 4/10 (10%) fetuses, in the absence of ultrasound markers that could address the diagnostic suspicion.

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P01.64D

Novel pathogenic splice variant in *PALB2* gene causing anemia Fanconi identified by transcriptomic analysis

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Fanconi anemia is rare congenital disease caused by mutations in genes responsible for DNA repair and expressing in chromosomal instability. Although the main symptom is anemia, clinical pattern differs in parents with mutations in genes according to different complementation group. The most severe clinical pattern is described in cases with *PALB2* gene mutations. These children develop severe anemia and early onset of different oncological diseases such as medulloblastoma, Wilms tumor, different leukemias. We report clinical case of a child with Fanconi anemia died because of medulloblastoma at the age 4 years 10 months. The disease was caused by frameshifting mutation in *PALB2* gene c. 172_175del inherited from mother and novel intronic deletion NC_000016.9:g. 23625423delAAAAATA inherited from father. Frameshift mutation was identified by exome sequencing of the affected child. The deletion was identified in father's blood by transcriptomic analysis. Functional analysis of mutation in minigene system confirmed its pathogenicity. As the family was interested in having a healthy child, understanding of molecular causes of disease in this family allowed to perform preimplantation genetic testing for monogenic disease (PGT-M). One IVF cycle was performed and 10 embryos were biopsied for PGT-M. According to the PGT-M results, it was determined that 3 embryos had both variants in heterozygose stage, 1 embryo inherited only c. 172_175del variant, 4 embryos inherited only NC_000016.9:g. 23625423delAAAAATA variant and 2 embryos did not inherit either of two variants. Preimplantation testing for aneuploidies was performed for these two embryos and they defined as euploid and were recommended for transfer.

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P01.66B

Preimplantation genetic diagnosis for chromosomal rearrangements using shallow whole genome sequencing at the blastocyst stage

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Preimplantation genetic diagnosis (PGD) for chromosomal rearrangements is used to avoid the transfer of embryos with genomic aberrations to the uterus and hence to improve implantation rate and avoid miscarriage or the birth of children with congenital anomalies. Currently, genomic microarrays are predominantly used for the detection of unbalanced structural abnormalities and aneuploidies in embryos from parents at risk. There are however several limitations to the use of microarrays such as constraints on resolution and throughput. With the advent of massive parallel sequencing (MPS), we investigated the use of shallow whole genome sequencing for PGD (CNVseq) on trophectoderm biopsies in our clinical diagnostic workflow. PGD was performed on embryos of translocation carriers in combination with vitrification and frozen embryo transfer in non-stimulated cycles. Data were collected from January 2016 onwards.

In total 45 PGD cycles have been performed for reciprocal (n = 39) and Robertsonian (n = 5) translocation and inversion (n = 1) carriers (total number of embryos =-185). Almost 60% of the analysed embryos showed chromosomal aberrations, which is in line with our earlier results with microarrays (Christodoulou et al., Fertilty&Sterility, 2017). The CNVseq protocol shows especially for small chromosomal segments better results than microarrays, and a resolution of ~5 Mb is achieved. Furthermore, many samples can be processed in batch leading to higher throughput. Besides abnormalities due to the parental rearrangement, also other chromosome abnormalities were detected in our cohort.

We describe the successful implementation of CNVseq on blastocysts in patients with a chromosomal rearrangement to identify euploid embryos for transfer.

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P01.67C

Whole-exome sequencing identifies novel causative variants and expands the phenotypic spectrum of PLK4-related primary microcephaly

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Loss-of-function variants in *PLK4*, encoding a key regulator of centriole duplication, cause autosomal recessive microcephaly and chorioretinopathy 2 (MCCRP2). Currently, only 13 cases from six families have been reported harboring four recessive variants. Following whole-exome sequencing analysis in 61 microcephalic cases, we identified novel causative *PLK4* variants in two aborted sib fetuses and an additional unrelated child. In the two fetuses, we found a nonsense variant and a serine substitution in compound heterozygous (CH) state, which the latter likely creates an additional phosphorylation site in the phosphodegron element of PLK4, leading to reduced protein level via accelerated autodestruction. Autopsy examination of the fetuses revealed white matter neuronal heterotopia and cerebellar vermis hypoplasia in one, and absence of corpus callosum in the other, apart from facial dysmorphism and microcephaly. Additional physical anomalies included 2-lobed right lung and accessory spleen, which have not been previously reported in MCCRP2. Furthermore, we identified (likely) pathogenic CH variants in the unrelated child, presenting with primary microcephaly, facial dysmorphism and moderate speech delay. Brain MRI showed simplified cortical gyri, dysplastic corpus callosum, and novel finding of large cerebellum-brain stem relative to the supratentorial region. Considering our cases and those previously reported, we consistently observed simplified gyri, abnormal corpus callosum and neuronal heterotopia, suggesting the importance of PLK4 in the regulation of neuronal migration. Moreover, we report a novel MRI finding as well as additional organ anomalies in MCCRP2, and describe the first deleterious missense variant located in the phosphodegron element outside the main PLK4 domains.

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P01.68D

Evidence for key roles of transcription factors and microRNAs in orchestrating placental gene expression patterns in common pregnancy complications

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Introduction: Approximately 5% of pregnancies suffer from pre-eclampsia (PE) and 3% of couples are affected by recurrent pregnancy loss (RPL).

Aims: To identify mechanisms affecting gene expression in common pregnancy complications PE and RPL.

Methods: We sequenced transcriptomes and miRNomes from term placental samples from normal (n = 8) and PE (n = 8) pregnancies and 1st trimester samples from 2 RPL cases and electively terminated pregnancies (ETP; n = 8). Differential expression was tested using DESeq and DESeq2. g:Profiler was used for enrichment analysis.

Results: In placentas of RPL cases, we detected 195 transcripts with altered expression in RPL compared to ETP (1). Over 60% of genes with altered expression in RPL possess binding sites for E2F transcription factors. E2F regulates the cell cycle and coordinates the mammalian endocycle and placental development.

Expression of 215 genes was altered in PE (2). Promoters of down-regulated genes (n = 173) exhibited strong enrichment for binding sites for transcription factors AP2, SP1 and LRF. Promoters of 77 genes (44.5%) contain potential response elements for all three transcription factors.

Correlation analysis between microRNA and gene expression identified an extensive network of coordinated expression involving multiple transcripts and microRNAs.

Conclusions: The E2F family of transcription factors represents a potential central coordinator of the shut-down of nuclear and cellular functions leading to fetal demise. Inadequate AP2, SP1 and LRF activity along with altered microRNA levels may drive gene expression changes in pre-eclampsia.

(1)Sõber et al. SciRep (2016): 38439.

(2)Sõber et al. SciRep (2015): 13336.

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P01.69A

Preimplantation genetic testing for cystic fibrosis and aneuploidy in clinical practice

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Introduction: Cystic fibrosis (CF) due to mutations in the CFTR gene is the most frequent reason for preimplantation genetic testing of monogenic disorders (PGT-M). Many women requiring PGD for CF are at advanced age that is a limiting factor of IVF success.

Materials and methods: We have performed PGT for CF in 81 IVF cycles for 51 couples between the years 2007

and 2017. The total number of examined embryos is 438. We have examined 317 samples of blastomeres from cleavage stage embryos and 121 samples of trophectoderm from blastocysts. The PGT-M for was performed by haplotyping using whole genome amplification (WGA) and multiplex fluorescence PCR analysis of short tandem repeat polymorphisms (STR markers) linked to the CFTR gene. We have recently added the aneuploidy detection (PGT-A) by NGS using Ion Torrent Proton as a second step in the evaluation of trophectoderm samples.

Results: We have amplified the DNA from 295 out of 317 single blastomeres (93%) and 104 out of 121 trophectoderm samples (86%). We have found 246 embryos not affected by CF (62%) or other abnormalities of chromosome 7 (monosomy, trisomy) using the haplotype analysis. We have further analysed the amplified DNA from 72 trophectoderm samples and detected aneuploidy in 31 out of them (43 %).

Conclusions: We have successfully used trophectoderm biopsy and whole genome amplification to combine the preimplantation genetic testing of a monogenic disorder (PGT-M) with aneuploidy detection (PGT-A) in clinical practice.

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P01.70B

Implementation of target capture enrichment on single and few cells for the robust detection of embryo abnormalities

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Introduction: High throughput non-invasive prenatal testing (NIPT) technologies have demonstrated safe, accurate and reliable results for the detection of fetal abnormalities, relying on the detection and analysis of cell free fetal DNA (cffDNA) in maternal plasma. However, such analysis is often limited by the low abundance of DNA, as in the case of fertilized embryos. Therefore, the development of novel, sensitive approaches which can provide reliable results from single/few cells is necessary.

Materials and Methods: Amplified DNA isolated from seven and 17 embryos was obtained from 3-day and 5-day biopsy cases. TArget Capture Sequences (TACS) were designed at a median resolution of 1Mb spanning all chromosomes and were used to perform in-solution hybridization capture enrichment as previously described[1]. Novel bioinformatics algorithms were also developed to determine the ploidy status of the samples.

Results: All samples were correctly classified and all abnormalities were detected including numerical and structural rearrangements. Results obtained were in agreement with array CGH.

Conclusions: Targeted sequencing is the preferred method for applications requiring high read depth. This assay in combination with a novel bioinformatics pipeline can be used for the genome-wide screening of fertilized embryos (PGS/PGD). It can also be used in cases where limited number of cells from affected tissues/individuals are available.

M. Ioannides: A. Employment (full or part-time); Significant; NIPD Genetics. **A. Achilleos:** A. Employment (full or part-time); Significant; NIPD Genetics. **K. Tsangaras:** A. Employment (full or part-time); Significant; NIPD Genetics. **C. Loizides:** A. Employment (full or part-time); Significant; NIPD Genetics. **P. Mina:** A. Employment (full or part-time); Significant; NIPD Genetics. **E. Kypri:** A. Employment (full or part-time); Significant; NIPD Genetics. **C. Sismani:** None. **G. Koumbaris:** A. Employment (full or part-time); Significant; NIPD Genetics. **P.C. Patsalis:** A. Employment (full or part-time); Significant; NIPD Genetics.

P01.71C

Various approaches to preimplantation genetic testing - experience from seven monogenic disorders

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Background: In the last decade preimplantation genetic testing (PGT) for severe genetic diseases emerged as a viable alternative to prenatal testing and termination of pregnancy for couples where one or both partners is a carrier or suffering from a debilitating genetic condition.

Materials & Methods: 7 couples opted for IVF-PGT after extensive genetic counseling. Indications were beta-thalassemia, epidermolysis bullosa, myotonic dystrophy type 1, Huntington disease, Fragile-X syndrome, hemophilia A and Duchenne muscular dystrophy. Trophectoderm biopsy of 32 5-day embryos was performed. DNA amplification was achieved by Repli-g (Qiagen). For all trinucleotide repeat disorders allele size was determined by fragment length analysis (TNR Diagnostics). RT-PCR was applied for epidermolysis bullosa and beta-thalassemia

(Microsynth), Sanger sequencing for haemophilia A and sex determination by aCGH for DMD. All protocols were tested in advance on donated unviable embryos.

Results: DNA from 31 embryos was available for testing after amplification (96,9%). DNA analysis was successful for all 31 embryos (100%). There were 21 unaffected embryos and 3 females (for the DMD case). Four of the couples (57,1%) achieved pregnancy after the first transfer, 2 couples (28,6%) after the second and only one (14,3%) did not get pregnant after three transfers, giving a 85,7% success rate of the IVF-PGT procedure. Except one pregnancy that has not yet reached time for prenatal confirmation, all PGT results were confirmed by DNA testing of CVS samples, 6 healthy babies were delivered.

Conclusion: IVF-PGT is a valid option for couples at risk to have a child with severe genetic condition.

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P01.72D

Preimplantation genetic testing for polycystic kidney disease is an option for affected families

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Introduction: In this study, preimplantation genetic testing (PGT) data for polycystic kidney disease (PKD) from 2005 until 2016 are reported. As males affected with autosomal dominant PKD (ADPKD) may present with reproductive system abnormalities and infertility, the clinical outcome was compared between couples with the female partner affected with ADPKD and couples with the male partner affected with ADPKD.

Materials and Methods: Sixteen single-cell clinical tests for PKD based on multiplex PCR of STR markers were applied for 91 PGT cycles for 43 couples.

Results: A diagnosis was obtained for 93.3% of the analysed embryos of which 36.8% were genetically transferable. Transfer of 74 embryos in 53 fresh cycles and transfer of 34 cryopreserved embryos in 33 frozen-warmed embryo transfer cycles resulted in a live birth delivery rate of 38.4% per transfer with 31 singleton live births, 2 twin live births and 1 ongoing pregnancy. The observed cumulative delivery rate was 57.8% per couple after five treatment cycles. The clinical pregnancy rate and live birth delivery rate was significantly lower for couples with the male partner affected with ADPKD compared with couples with the female partner affected with ADPKD. However, female age was the only variable significantly associated with live birth delivery rate.

Conclusions: This study shows that PGT for PKD performed in our centre offers good reproductive outcomes from both fresh and frozen embryo transfers. Males affected with ADPKD who suffer from infertility should be advised to seek treatment on time to improve their chances of conceiving a child.

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P01.73A

DNA copy number variations in a cohort of 216 Italian women with premature ovarian failure

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Premature ovarian failure (POF) is considered as a multifactorial and heterogeneous condition affecting approximately 1% women of reproductive age. Despite the extensive research, the considerably complex pathogenesis of POF is still not well understood. POF can develop as result of a broad spectrum of pathogenic mechanisms including genetic, autoimmune and iatrogenic causes that leads to follicular dysfunction or depletion. In recent years, many research studies are trying to find out the genetic component of the disease by using different high-resolution methods. We have performed a case-control genetic association study, using high-resolution SNP microarrays to investigate DNA copy number variations (CNVs) of 216 Italian women presenting POF phenotype and 240 women from the Italian general population as a control. All patient and control samples were collected at the Division of Genetics and Cell Biology, San Raffaele Scientific Institute and University of Milan, Italy and genotyped by Illumina

PsychArray BeadChips at the Estonian Genome Center University of Tartu Genotyping core in Tartu, Estonia. Microarray data, analyzed using different algorithms, revealed both, genomic regions containing genes previously associated with POF (e.g. 26.8Mb 1q41-q44 duplication affecting *FMN2* gene) and novel potentially clinically significant CNVs (e.g. 11p15.2 microdeletion). In addition to autosomal CNVs, we identified several POF critical regions on the X chromosome. The currently known POF genes only account for a small proportion of patients, while the majority remain without a genetic diagnosis. Using whole-genome DNA microarrays, the present study provides novel insight into the implications of CNVs in genetic aetiology of POF.

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P01.74B

The diagnostic yield of exome sequencing in the prenatal setting: A clinical laboratory experience

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Introduction: Fetal ultrasound abnormalities pose a unique diagnostic challenge. In order to help increase the diagnostic yield of underlying genetic etiologies exome sequencing (ES) is gaining use in the prenatal setting.

Methods: We retrospectively analyzed ES results on 233 deceased fetuses and 33 ongoing pregnancies with ultrasound anomalies.

Results: Of the 233 deceased fetuses, 76% of testing was performed as proband-parent trios. Fifty-six percent were male and 44% female. Most cases had multiple congenital anomalies (MCA) (78%). The most common ultrasound findings were central nervous system (CNS) anomalies (51%), hydrops (33%), skeletal abnormalities (30%), cardiovascular defects (28%), neuromuscular findings (26%), and genitourinary anomalies (24%). A definitive molecular diagnosis was identified in 28% of cases, a possible diagnosis in 36%, only a candidate gene was reported in 10%, and 26% of cases had no reportable variants. We also analyzed 33 prenatal specimens from ongoing pregnancies. All cases were trios and had non-diagnostic standard genetic testing prior to ES. Seventy-three percent were male and 27% female. Most cases had MCA (79%). Common ultrasound findings included cardiovascular anomalies (33%), genitourinary abnormalities (33%), CNS defects (30%), and hydrops (30%). For all ongoing pregnancies, a definitive molecular diagnosis was identified in 30.3% of cases, a possible diagnosis in 33.3%, only a candidate gene was reported in 6.1%, and 30.3% had no reportable variants.

Conclusion: Although interpretive and ethical issues remain a concern in the prenatal setting, ES may identify genetic variants responsible for fetal anomalies and impact prognosis, medical management, and recurrence risks.

A. Telegrafi: A. Employment (full or part-time); Significant; GeneDx. **C. Yates:** A. Employment (full or part-time); Significant; GeneDx. **K.G. Monaghan:** A. Employment (full or part-time); Significant; GeneDx. **E. Ryan:** A. Employment (full or part-time); Significant; GeneDx. **B. Friedman:** A. Employment (full or part-time); Significant; GeneDx. **H. Sroka:** A. Employment (full or part-time); Significant; GeneDx. **R. Willaert:** A. Employment (full or part-time); Significant; GeneDx. **R. Chikarmane:** A. Employment (full or part-time); Significant; GeneDx. **J. Juusola:** A. Employment (full or part-time); Significant; GeneDx.

P01.76D

Prenatal array CGH: comparison of 400kb and 3Mb resolution

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There is currently no clear consensus on the cost-benefit of detailed genome-wide copy-number analysis for pregnancies with ultrasound anomalies. Decisions regarding test resolution should consider evidence concerning diagnostic yield, cost (technical and analytical), reporting times and the number of uncertain and incidental findings. From 2012 to 2017 we investigated pregnancies with ultrasound anomalies using a prenatal array testing strategy designed to minimise uncertain results and incidental findings whilst identifying clinically significant abnormalities; a 3Mb backbone resolution was supplemented with high resolution analysis of 23 regions known to be associated with severe, fully penetrant syndromes. Review of 480 of these cases (after anonymization) at an average of 120kb resolution found no severe, fully penetrant imbalance had been missed. European guidelines now recommend a genome-wide resolution of at least 400kb (the evidence base for this guideline is unclear). To comply with this, in 2017 we introduced a 400kb analysis strategy, with high resolution analysis of 25 syndrome regions; only findings associated with the ultrasound anomalies and actionable or severe, early-onset incidental findings were reported. Of 201 prenatal samples tested, 22 were reported as abnormal, including one incidental finding, 178 as normal and one

failed. Only one case (0.5% of samples), a de novo 1.6 Mb 15q25 deletion (OMIM 614294) would not have been identified using our previous analysis strategy. Fourteen unique imbalances (7% of samples) were not reported following detailed variant classification; in addition, four susceptibility loci (class 4 variants) were not disclosed. A cost-benefit comparison of these strategies will be discussed.

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P01.77A

Case report: Gonosomal placental mosaicism leads to a false-positive NIPT result

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Introduction: A 37 years old pregnant woman with no fetal ultrasound abnormalities received a NIPT result at early gestational age indicating a monosomy X. For confirmatory purpose, amniocentesis including aCGH was performed: the fetal karyotype was reported to be normal, male (46,XY) instead of the expected 45,X. Perinatally, another NIPT with high sequencing depth was requested (Prenatalis®, MVZ Martinsried) in concert with postpartal FISH of placental villi to shed light into these discordant results.

Method: Prenatalis® was performed with ~ 21 million reads used for detection of aneuploidy 13, 18, 21, X and Y. Postpartal FISH analysis was done on nuclei of placental villi by using the centromere specific DXZ1-, DYZ3- and D18Z- probes (Cytocell, Cambridge, UK).

Results: Prenatalis® confirmed the initial monosomy X result at a fetal fraction of 39% (GA:36+6). FISH-analysis revealed placental mosaicism with a dominant X0-cell line (86% of all nuclei) in combination with a XY cell line (10%). 4% of nuclei showed two X-chromosomes, probably a contamination of maternal cells. The patient delivered a phenotypically normal, male baby, who was not karyotyped further.

Conclusion: The results presented above describe confined placental mosaicism of a dominant monosomy X cell line in concert with a low level XY-cell line. It can be deduced from the NIPT results, that the placenta released cfDNA exclusively from 45,X loci, since y-chromosomal cfDNA could not be detected during NIPT. Since placental cfDNA only serves as a proxy for the fetus, confirmation of positive NIPT results are highly recommended.

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P01.78B

Rapid whole exome sequencing; implementation in the prenatal setting

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The introduction of whole exome sequencing (WES) in genome diagnostics has dramatically changed the current practice in clinical genetics. It is expected that WES and ultimately whole genome sequencing will replace current routine clinical practice (array based technologies), not only after birth but also during pregnancy. The implementation of WES in a prenatal setting for genetic analysis of fetuses with multiple congenital abnormalities has the potential to increase the diagnostic yield and thereby improving prognostic information for professionals and expectant parents. However, there are a number of factors that need to be taken into account before WES could be part of the prenatal diagnostic workup. These include technical and practical aspects like long turn-around-times (TATs), costs, difficulties in interpreting variants and limited possibilities to determine the fetal phenotype. Intensive collaboration within a multi-disciplinary team consisting of a molecular laboratory specialist, a clinical geneticist and a fetal medicine specialist is required. Furthermore, the possibility of detecting variants of unknown significance (VOUS) or incidental findings (IF) may lead to ethical dilemmas and demands careful pre- and post-test counselling.

Until now, rapid WES with short TATs has been performed on a case-by-case basis in our centre in a small number of pregnancies in the second or third trimester. We will provide an overview of the workflow, the challenges and the difficulties encountered. To warrant an accurate, more widespread implementation of prenatal WES, there is a strong need for clear criteria, data-sharing and an (inter) national guideline.

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P01.79C

Multilevel regression modeling improves STR classification in QF-PCR analysis for common fetal chromosomal aneuploidies

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The quantitative fluorescent polymerase chain reaction (QF-PCR) has proven to be a reliable method for detection of common fetal chromosomal aneuploidies, with advantages over conventional karyotyping such as cost-effectiveness, reliability and requirement of only small amount of material. However, there are some technical shortcomings, involving the necessity to perform two or more multiplex PCR reactions simultaneously for a given sample or the uncertainty of aneuploidy determination when the STR (short tandem repeats) height ratio is unusual due to large size difference between alleles. Here, we present an in-house one-tube multiplex QF-PCR method including 20 PCR markers (14 STR markers and 6 fixed size) for rapid prenatal diagnosis of chromosome 13, 18, 21, X and Y aneuploidies. In order to improve the aneuploidy classification of a given diallelic STR marker, we used a total of 7630 diallelic genotypes (88 trisomic and 7542 normal) from 871 samples to employ multilevel logistic regression analysis using "height ratio" and "allele size difference" as fixed effects and "marker" as random effect. We employed two regression models, one for the 2:1 height ratio ($n = 47$) and second for the 1:2 height ratio ($n = 41$) of the trisomic diallelic markers. Both models achieved 100% specificity for marker aneuploidy classification on training data as compared to 98.3% (2:1 ratio) and 97.9% (1:2 ratio) specificity when using only height ratio for classification. In conclusion, adjusting for the allele size difference and marker type improves the STR classification, eliminates sample re-testing and reinforces the robustness of the QF-PCR method for prenatal testing.

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P01.80D

High-resolution array-CGH analysis and Targeted Whole Exome Sequencing on patients affected by Primary Ovarian Insufficiency (POI) identified new genes involved in oocyte grow and differentiation

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POI is a heterogeneous group of disorders that affect women fertility whose genetic origin has been clarified in less than 30% of cases. To unveil new causative genes essential for ovarian function we searched for rare high-penetrance Copy Number Variants (CNVs) in a cohort of 67 46,XX patients affected by the most severe phenotype (primary amenorrhea). High-resolution array-CGH analysis detected 72 rare CNVs according to the Database of Genomic Variants in 49 patients. CNVs gene content analysis and disease prioritization selected 37 CNVs involving 2 POI-associated genes and 42 putative candidate genes (e.g. *TP63*, *VLDLR*). The research of this CNVs in an *ad-hoc* cohort of 134 control women supported their actual rarity. Despite the presence of ovary genes also in the *ad-hoc* cohort, chi-q and Wilcoxon tests showed in patients a significant enrichment of ovary-related CNVs/genes (*P=0.0132/P=0.0126*) supporting array-CGH as a valuable tool in identifying novel POI molecular defects. Array-CGH genes identified together with their predicted interactors, and other known ovary/POI-related genes ($n = 226$) were then screened in a targeted-WES analysis of 102 secondary amenorrhea patients. After filtering variants (MAF<0.005; LoF SNVs inclusion) a total of 375 possibly pathogenic SNVs were found in 31 array-genes, 12 interactor-genes, and 83 ovary/POI-related genes. Burden test analysis versus 1000G_EUR control women confirmed a statistical significance for 1 interactor-*TP63* gene (*P=1.65E-07*) and 2 ovary/POI-related genes (*FSHR*, *P=1.66E-05;FOXO3, P=7.31E-05*). This combined approach allowed to increase the knowledge about POI pathogenesis and will probably provide the basis for a more accurate genetic diagnosis of POI patients.

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P01.81A

Genetic analysis of products of conception of couples with recurrent miscarriage using QF-PCR and array-CGH testing strategy

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Introduction: Although previous studies have shown that embryonic chromosome aberrations are the most common cause of recurrent miscarriage (RM), the comprehensive genetic evaluation using the combination of quantitative fluorescence-polymerase chain reaction (QF-PCR) and array-comparative genomic hybridisation (aCGH) was not used systematically for their detection in the clinical setting. We aimed to investigate the frequency and type of chromosome aberrations in POCs of couples with at least one previous miscarriage using the QF-PCR and a-CGH strategy.

Materials and Methods: This retrospective study was conducted on 73 first-trimester POCs (September 2014-February 2017). The POCs were collected from 73 women with at least one previous miscarriage and analysed for chromosomal anomalies using QF-PCR and aCGH as part of the routine clinical evaluation.

Results: Chromosome aberrations were detected in 52/73 POCs (71.2%), of which 41 (56.2%) were identified by QF-PCR and an additional 11 (15.1%) by aCGH. Numerical aberrations constituted the majority (92.3%) of abnormalities, with trisomies as the most common subtype (72.9%). Causative structural aberrations were found in three samples (5.8%) and variant of unknown significance in one sample. The frequency of chromosome aberrations was not dependent on the number of previous miscarriages, whereas it significantly increased with advanced maternal age.

Conclusions: The results of our comprehensive genetic analyses of POCs of RM couples confirm the QF-PCR and aCGH combination as an effective diagnostic strategy. Considering the high frequency of chromosome aberrations, a routine genetic analysis of POCs should be considered, which could improve the clinical approach to couples with miscarriage.

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P01.82B

Telomere shortening as the main indicator of non-viable fetus elimination

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Background: Telomeres are transcriptionally inactive genomic areas, which, if shortened, are associated with pathological processes, unsuccessful fertilization, aging, and death. Telomere dysfunction has also been linked to chromosomal rearrangements and genomic instability. The role of telomeres in postnatal life has been extensively studied and discussed both in physiological as well as in pathological processes. However, the role of telomere length in prenatal development is still poorly understood, and mainly concerns the preimplantation stage. The aim of this study was to estimate relative telomere length in spontaneously eliminated human embryos between 5th and 12th week of gestation.

Results: Relative telomere length was measured from total genomic DNA using a real-time polymerase chain reaction approach. In this study, we examined relative telomere length in 80 spontaneously eliminated embryos and in 25 embryos eliminated due to induced abortions. Relative telomere length in spontaneous abortions was significantly lower ($P = 0.000001$) compared to the induced abortions. Spontaneous abortions with aneuploid anomalies (monosomy X, trisomy 21, trisomy 16 and triploidy) were characterized by shorter telomeres, compared to spontaneous abortions, subgroup with euploid (46,XN) karyotype.

Conclusion: Spontaneously lost pregnancies are characterized by shortened telomeres, especially in embryos with aneuploidies. We hypothesize that the shortening of telomeres is involved in the processes leading to spontaneous abortions.

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P01.83C

Targeted cfDNA Analysis Using DANSR assays for Determination of Fetal *RHD* Status

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Objectives: To develop a targeted cell-free (cfDNA) test, the Harmony® prenatal test, enhancement that allows determination of fetal RhD status in RhD-negative pregnant women.

Method: 12 simulated pregnancy plasma samples with known *RHD* genotype were prepared by titrating non-pregnant, *RHD*-positive cfDNA (fetal source) into non-pregnant, female *RHD*-negative cfDNA (maternal source) to simulate fetal fractions of 5%, 10% and 15%. A 0% sample served as a negative control. Digital Analysis of Selected Regions (DANSR) assays targeting exons 2, 3, 4, 5 and 7 of the *RHD* gene were added to existing DANSR assays and the generated DANSR products were hybridized onto a custom DNA microarray for analysis of fetal fraction and determination of fetal *RHD* status using the fetal fraction optimized algorithm FORTE.

Results: In all 12 simulated pregnancy samples, *RHD* sequences were detected. The *RHD* signal in each case correlated with fetal fraction and was therefore consistent with an *RHD*-positive fetal source on the background of an *RHD*-negative maternal source. As expected, no *RHD* sequences were detected for samples with 0% fetal fraction.

Conclusion: Targeted cfDNA testing using DANSR assays has the potential to determine fetal *RHD* status and be used as a noninvasive screening method to identify pregnancies at increased risk for RhD immunization.

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P01.84D

Prenatal diagnosis of mosaic ring chromosome 16 - a rare event with uncertain prognosis

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Ring chromosomes are rare cytogenetic findings (prenatal frequency ~ 0.0075%) often associated with an abnormal phenotype, depending of the chromosomal origin, genetic content and the presence of a mosaic. Supernumerary ring chromosome 16 [r(16)] is rarely observed and mosaicism makes the genotype/phenotype correlation difficult.

We report a de novo mosaic r(16) detected after prenatal diagnosis in a woman referred for advanced maternal age. Multiplex ligation-dependent probe amplification (MLPA) for aneuploidy testing of chromosomes 13, 18, 21 and X was normal. Karyotype was 47,XX,+r[10]/46,XX[15]. Chromosomal microarray analysis (CMA) on DNA obtained from long-term cultured amniocytes did not detect any alterations. MLPA with a pericentromeric probe kit on an uncultured sample showed a chromosome 16 gain, encompassing 16p11.2 and 16q11.2 regions, including *TGFB1I1*, *AHSP*, *VPS35* and *ORC6* genes, leading to partial characterization of the r(16). Although no phenotype has been correlated with overexpression of these genes, the 16p11.2 region is associated with neurodevelopmental disorders. Nevertheless individuals with microduplication of 16p11.2 and normal development have been described.

The lack of a precise definition of genetic content of the r (16) and its mosaic form leads to uncertain prognosis of clinical outcome.

After genetic counseling the couple opted to continue the pregnancy. At birth no major malformations were observed and a lower level of mosaic r(16) was observed in peripheral blood.

The mosaicism, as well as limitations of CMA in those cases, prevent a refined characterization of these genomic imbalances and pose a challenge in genetic counseling.

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P01.85A

A novel partial deletion of the *NR5A1* gene in a female patient with 46, XY disorder of sex development

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NR5A1 (Steroidogenic factor 1, SF-1) is a transcriptional regulator of genes required for normal adrenal and gonadal development and function. Mutations in *NR5A1* have been identified in patients with various forms of disorders of sex development (DSD), including gonadal dysgenesis with or without adrenal insufficiency. To date microdeletion or partial deletion involving the *NR5A1* gene have been reported in only a few of cases with DSD.We present a patient with female external genitalia, clitoromegaly, bilateral ingiunal hernia containing testicles, mixed internal genitalia (uterus, Fallopian tube, epididymis), minor facial dysmorphism, normal adrenal function, low testosterone, high FSH levels. Family history is negative for disorders of sex development or premature ovarian failure. Chromosome analysis revealed a 46,XY karyotype. Array CGH did not detect pathogenic copy number variations. Next generation sequencing of the most common DSD genes did not identify pathogenic variants. Genomic DNA was screened for small deletion/duplication of genes commonly affected in DSD using the SALSA Intersex MLPA kit (MRC-Holland) according to manufacturer's protocol. We identified a novel partial deletion encompassing the exons 5 and 6 of the *NR5A1* gene leading to haploinsufficiency of the gene, that is alone sufficient to cause the patient's abnormal sexual development.This report expands upon the range of mutations associated with *NR5A1* gene, further confirms the role of *NR5A1* deletions in 46,XY DSD and emphasises the utility of MLPA as a genomic screening tool in the workup of DSDs of unclear etiology.This study was supported by Ministry of National Economy, Hungary GINOP-2.3.2-15-2016-00039

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P01.87C

A novel next generation sequencing assay for the detection of SMA carrier status and the number of copies of *SMN2*

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Spinal muscular dystrophy (SMA) is an inherited autosomal recessive neuromuscular disease caused by a defective *SMN1* gene. *SMN1* codes for a protein involved in RNA processing. The disease affects 1 out of 6000 newborns, while about 1 out of 50 individuals are carriers.

SMN1 has a paralog *SMN2*, which differs in coding sequence by just 1 base. *SMN2* produces a functional protein though less efficiently than *SMN1*. These genes are located 500 kb apart on chromosome 5, which permits frequent recombination events that result in deletions, duplications or chimeras. Evolution has selected for multiple copies of *SMN2* since having several copies can partially compensate for a non-functional *SMN1* gene. Because of the severity of SMA, carrier and newborn screening is important. To be utilized in carrier screening, an assay has to detect one copy of *SMN1* with 100% sensitivity, which necessitates distinguishing every *SMN1* copy from *SMN2*.

An NGS assay was developed to detect one copy of *SMN1* and the number of *SMN2* copies in SMA carriers in an easy and scalable protocol suitable for automatization. The assay is performed in one tube, using a ligation-free method. Testing of clinical research samples with known numbers of *SMN1* and *SMN2* has demonstrated the precision of the assay to detect the number of *SMN* copies. The protocol can also be easily expanded to include all *SMN1* mutations and/or be combined with assays to detect variants in other known carrier-screening genes for future clinical research.

Research use only. Not for diagnostic use.

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P01.88D

A new candidate biomarker in Spermatogonial Stem Cell maturation : Dynamin 2

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Dynamin 2 (DNM2) belongs to the GTPase superfamily. Beside having important roles in the regulation of membrane fission and fusion events dynamins induce differentiation of germ cells in spermatogenesis.

In this study, our aim was to investigate dynamin 2 gene expression and the relationship between this biomarker with impaired spermatogenesis in non-obstructive azoospermia (NOA) and obstructive azoospermia (OA) as control group. NOA group consisted of 20 hypospermatogenesis (HS), 20 maturation arrest (MA), 20 Sertoli Cell Only syndrome (SCO) patients. The biomarker was analyzed by RT-PCR array.

When compared with the control group, DNM2 gene expression in HP, MA and SCO groups showed 1.60 ± 0.30 ($p > 0.05$), 0.28 ± 0.05 ($p < 0.001$) and 0.86 ± 0.18 ($p > 0.05$) fold changes, respectively.

The dynamin family of proteins has important regulatory roles in membrane remodelling and endocytosis. By this way they also affects the cell viability. Thus, the decrease in DNM2 gene expression in MA group suggests that clinically DNM2 expression deficiency may cause maturation arrest. DNM2 is thought to be an important marker for the understanding the etiology of male infertility and in the development of treatment protocols for azoospermic patients with MA.

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P01.89A

Expression profiles of Spermatogonial Stem Cells' marker genes in azoospermic patients with different pathologies

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Spermatogonial stem cells (SSCs) have major roles on spermatogenesis and male fertility. Identification of SSCs and determination of their effects on impaired spermatogenesis are very important for elucidation of the etiology of male infertility. In this study, our aim was to investigate CD90, CD29, CD49f and POU5F1 genes expressions and the relationship between these biomarkers with impaired spermatogenesis in non-obstructive azoospermia (NOA) and obstructive azoospermia (OA) as control group. NOA group consisted of 20 hypospermatogenesis (HS), 20 maturation arrest (MA), 20 Sertoli Cell Only syndrome (SCO) patients. The biomarkers were analyzed by RT-PCR array. All groups were compared with the control group. CD29 and CD49f gene expressions showed 0.08 ± 0.01 and 0.65 ± 0.15 fold decreases ($p < 0.05$), in the HS group, while CD90 and POU5F1 genes showed 1.20 ± 0.21 and 0.83 ± 0.24 fold changes, respectively ($p > 0.05$). In the MA group, CD90, CD49f and POU5F1 gene expressions showed 0.40 ± 0.05, 0.22 ± 0.03 and 0.16 ± 0.02 fold decrease ($p < 0.05$) respectively, while CD29 gene showed 0.85 ± 0.10 fold change ($p > 0.05$). In the SCO group, CD90, CD29 and POU5F1 gene expressions showed 4.73 ± 1.03, 2.10 ± 0.47 and 3.56 ± 0.68 fold increases, ($p < 0.05$) respectively, while CD49f gene expression showed 1.67 ± 0.37 fold change ($p > 0.05$). As a conclusion, it was found that SSCs biomarkers exhibit different profiles in NOA patients. We believe that the curative protocols which will be developed against the effects of these markers in spermatogenic defects will be very valuable in terms of human reproductive health.

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P01.90B

Robust preimplantation genetic diagnosis of spinal muscular atrophy combining triplex *SMN1* detection with multi-microsatellite haplotyping

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Background: Preimplantation genetic diagnosis (PGD) of spinal muscular atrophy (SMA) is vulnerable to misdiagnosis arising from allele drop-out or exogenous DNA contamination. We describe a PGD strategy that detects SMA with high diagnostic confidence and accuracy.

Methods: A triplex PCR-minisequencing assay was developed for reliable detection of *SMN1* and *SMN2*. A single-tube assay was developed to simultaneously amplify 13 highly polymorphic microsatellite markers located within 0.5 Mb of the 1.7 Mb duplicated *SMN* region on chromosome 15q13.2. Single cells were subjected to whole genome amplification, and separate aliquots were used for triplex *SMN1/2* detection and for tridecaplex microsatellite-based haplotyping. The strategy was validated on 24 single cells isolated from cell lines of an SMA case-parent trio.

Results: Triplex *SMN1/2* PCR-minisequencing reliably detected single-copy *SMN1* even in the presence of five *SMN2* copies. Only *SMN2* was detected in all SMA-affected samples. Observed heterozygosities of the 13 flanking microsatellite markers ranged from 0.56 to 0.8, and 98.4% of genotyped individuals were heterozygous for 2 or more markers on either side of *SMN1*. Triplex *SMN1/2* PCR-minisequencing results were completely correlated with observed marker diplotypes in all tested samples.

Conclusion: Triplex detection of *SMN1* and *SMN2*, combined with linked multi-marker diplotyping, improves diagnostic confidence for SMA PGD. The highly polymorphic tridecaplex microsatellite panel can potentially be informative in most if not all at-risk couples.

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P01.91C

Numerical and structural genomic aberrations in spontaneous abortions, detected by array CGH analysis

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Background: Chromosomal abnormality in the product of conception is the reason for 50-70% of abortions. Chromosomal microarray analysis of fetal tissue has been proposed as a technique to evaluate the cause of isolated and recurrent early pregnancy loss (miscarriages) and later pregnancy loss (intrauterine fetal demise).

Materials and Methods: In this study, we applied array CGH method for analysis of chromosomal imbalances at a high resolution in 24 samples of spontaneous abortions.

Results:

Genomic aberration	%
Trisomy 22	8
Trisomy 16	8
Monosomy X	8
Trisomy 18	4
Trisomy 19	4
Trisomy 20	4
Trisomy 21	4
Trisomy 15	4
Double trisomy 18 and 19	4
Duplication 7p	4
Tetrasomy 9p	4
Chromotripsis	8
Euploid	**36**

Conclusion: In 15 of 24 spontaneous abortions (64%), genomic anomalies were discovered by array CGH analysis. Two thirds of them could be detected by the rapid DNA analysis (offered in our country as a rapid QF-PCR analysis for aneuploidies 13, 15, 16, 18, 21, 22, X and Y). Numerical anomalies were detected in 73% of aberrant cases, and in 27% - structural aberrations/chromothripsis were revealed. For couples with recurrent pregnancy loss and evidence of a structural genetic abnormality in one of the parents, preimplantation genetic diagnosis with transfer of unaffected embryos or the use of donor gametes might be considered for therapy.

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P01.92D

Phenotype variability of NR5A1 (SF1) gene mutations in DSD patients

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Background: NR5A1(Steroidogenic Factor 1, SF1) gene mutations result in various forms of disorders of sex development (DSD), adrenal insufficiency, primary hypogonadism, male and female infertility.

The Aim: To evaluate the clinical variability of ambiguous phenotypes and the gender assignment in DSD patients with SF1 mutations.

Materials and Methods: Clinical examination, hormonal tests, ultrasound, laparoscopy, standard cytogenetic examination, and DNA analyses, including Sanger and next-generation sequencing.

Results: Case 1. A 46,XY female patient, aged 18 months with clitorophallus. A small testis was detected in the right labioscrotal fold. Hormonal tests showed LH - 0.25 IU/l, FSH - 12.9 IU/l, and testosterone after hCG stimulation - 0.3 nmol/l. Pelvic ultrasonography and laparoscopy showed the uterus, fallopian tubes and dysgenetic abdominal gonad on the left side. DNA analysis detected the heterozygous nonsense mutation c.256delA of the NR5A1 gene. Case 2. A 46,XY boy, aged 5 months, with undermasculinized Prader III genitalia, perineal hypospadias and small testes in bifid scrotum. Uterus was not found. Hormonal tests showed testosterone - 5.6 nmol/l, LH - 2.2 IU/l, FSH - 5.0 IU/l, and AMH - 43.8 ng/ml. DNA analysis found heterozygous p.R313C mutation of the NR5A1 gene.

Conclusions: The 46,XY patients with heterozygous NR5A1 mutations presented various phenotypes of gonadal development. The patient with nonsense mutation presented female phenotype with gonadal dysgenesis and Müllerian derivates. The male patient with NR5A1 missense mutation developed genitalia ambiguous with moderated

undermasculinization and no Müllerian structures, that is characteristic for partial 46,XY gonadal (testicular) dysgenesis.

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P01.93A

Higher levels of circulating mRNA for Tenascin X (TNXB) gene in maternal plasma at the second trimester of pregnancy in isolated congenital ventricular septal defects

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Introduction: Maternal plasma is a source of circulating placental nucleic acids; therefore it is a powerful tool for prenatal screening for congenital heart diseases (CHD). Previous studies showed that the identification of circulating mRNAs of MAPK1, IQGAP1 and Visfatin in maternal plasma had different gene expressions between CHD foetuses and control foetuses. This method allowed us to further investigate the expression of Tenascin X gene (TNXB) published as involved in CHD. Levels of circulating mRNA for the TNXB were investigated in pregnancies with ventricular septal defects during 2nd trimester of pregnancy.

Materials and Methods: The circulating mRNA assay was isolated from blood samples collected in several Institutes in Italy from March 2016 to July 2017. TNXB gene expression was investigated by RT-PCR in 10 women carrying foetus with ventricular septal defects and 31 controls at 19-24 weeks of gestation.

Results: RT-PCR showed that TNXB gene expression was higher in foetuses with ventricular septal defects with a $2^{-\Delta\Delta CT}$ value of 4.38 ± 3.01 vs 1.00 ± 0.80 in controls. CSH_2 was used as housekeeping gene which expression value was normalised based on several gestational ages.

Conclusions: The data confirmed a link between circulating mRNA and CHD; ventricular septal defects could be associated with abnormal level of TNXB mRNA during the 2nd trimester of pregnancy. In future, the variation of TNXB gene expression will be used to identify the foetuses with CHD, however this molecular marker will be assessed throughout prospective studies in a wider population.

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P01.94B

Genetics of sex hormone-binding globulin and testosterone levels in fertile and infertile men of reproductive age

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Introduction: Testosterone (T) is a central androgenic hormone. Sex hormone-binding globulin (SHBG) modulates T bioactivity. The current understanding of genetic variation contributing to male reproductive hormone levels is moderate. We aimed to confirm or reject genetic associations of top-loci (*SHBG*, *GCKR*, *SLCO1B1*, *JMJD1C*) from GWA-studies for SHBG and T, and uncover additional genetic effects on male fertility-related parameters (J Endocr Soc. 2017 1(6): 560-576).

Material and Methods: Study groups: young men (n = 540; 19.3 ± 1.8 years), severe idiopathic male infertility patients (n = 641; 31.6 ± 6.0 years), male partners of pregnant women (n = 324; 31.9 ± 6.6 years). Recruitment: Andrology Unit, Tartu University Hospital, Estonia. Analysis: genetic associations with reproductive parameters (linear regression, meta-analysis).

Results. Robust associations with SHBG for *SHBG* rs1799941 (meta-analysis: P=3.7x10^{-14}; beta = 4.67(0.62) nmol/L), *SHBG* rs727428 (P=7.3x10^{-11}; -3.74(0.57), *SHBG* Pro185Leu (rs6258; P=1.2x10^{-4}, -12.2(3.17) and *GCKR* Pro446Leu (rs1260326; P=1.5x10^{-4}; -2.2(0.59). Total T correlates with genetically modulated SHBG levels (r=0.48-0.74, $P < 0.0001$), guaranteeing stable availability of free T. Among infertile men, *SHBG* Pro185Leu shows downstream effect on LH (P=5.1x10^{-5}; -1.66(0.57) IU/L) and FSH (P=3.4x10^{-3}; -2.48(1.23) IU/L). No associations for *SHBG* Asp327Asn (rs6259), *SLCO1B1* Val174Ala (rs4149056), *JMJD1C* rs7910927.

Conclusions. We replicated previously reported associations and detected additional effects for four of seven analysed GWAS hits. These variants are promising candidates for the future studies of hypotestosteronemia in aging men, as well as promising pharmacogenetic targets for hormone replacement therapy in males with fertility problems.

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P01.95C

Evaluation of an in-house-developed HRM based approach for Non-invasive prenatal diagnosis of beta thalassaemia

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Introduction: Thalassaemias are the most common monogenic disorders worldwide. In the majority of cases prenatal diagnosis of embryos at risk is performed through an invasive procedure that has 1-2% probability of miscarriage. We have previously developed a non-invasive prenatal testing (NIPT) approach, based on analyzing free fetal DNA. Here, we present the results of 32 cases where the parental beta globin gene mutations are inherited by the fetus. Our method is based on High Resolution Melting (HRM) analysis and covers all possible combinations of fetal inheritance.

Materials and Methods: ffDNA is isolated from 2ml maternal plasma and screened for the presence of β globin gene parental mutations based on an in-house developed HRM approach. Previously described SNPs in the vicinity of β globin gene, for which parents presented distinct haplotypes, were also determined and used for identification of the fetal fraction. Sex determination was also helpful in case of male embryos.

Results: Our hitherto results (32 cases) cover all possible combinations like male or female embryos that inherited either the paternal or the maternal mutation and cases where the embryo has inherited both parental mutations. Our results succeeded in obtaining the same diagnosis with the preceded analysis of corresponding chorionic villus sampling, showing up to now 100% diagnostic capacity.

Conclusions: The described approach, after further evaluation, may allow its application at the diagnostic level as a primary or secondary method for NIPT for beta thalassaemia and if accordingly adopted it may be used for other monogenic diseases as well.

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P01.96D

Performance of the VeriSeq NIPT Solution - a novel PCR-free, paired-end sequencing-based noninvasive prenatal screening test

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Objectives: To develop and evaluate performance of a novel and highly automated, paired-end sequencing-based noninvasive prenatal test (NIPT) for fetal chromosomal aneuploidies on chromosomes 21, 18, 13, X, and Y.

Methods: We developed a PCR-free, paired-end sequencing-based NIPT, the VeriSeq™ NIPT Solution, that estimates fetal fraction (FF) and screens for fetal chromosomal aneuploidies in maternal plasma samples. Performance of VeriSeq NIPT Solution was determined by analyzing 3057 frozen maternal plasma samples that had been tested previously using a single-end sequencing-based NIPT (Verifi™ Prenatal Test, Illumina, Inc.); samples were blinded prior to reanalysis. Clinical outcomes (cytogenetic analysis or newborn physical examination) were available for all cases used to determine assay sensitivity and specificity.

Results: At a sequencing depth of 8M reads on a NextSeq™ 500 or 550, the limit of detection was below 3% FF for each chromosome-of-interest. Test performance was determined using 3107 samples, of which 21 (0.7%) were not reported because of QC failure on the only plasma aliquot available. Sensitivity was 98.9% (90/91), 90.0% (18/20), and 100% (8/8) for trisomy 21, trisomy 18, and trisomy 13, respectively. Specificities were ≥99.9% for all three trisomies; there were 8 false-positive results (one trisomy 21, three trisomy 18, and four trisomy 13). Concordance for sex chromosome aneuploidies ranged from 80.0-100%.

Conclusions: VeriSeq NIPT Solution showed good test performance for the detection of fetal chromosomal aneuploidies, even at low fetal fractions. This paired-end sequencing-based NIPT assay detects fetal chromosomal aneuploidies with high sensitivities and specificities and a low assay failure rate.

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P01.97A

The compare results of different whole genome amplification methods for purposes of cell-based noninvasive prenatal diagnosis

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Introduction: Trophoblast cells in blood of pregnant women are a potential target for non-invasive prenatal testing, but the number of trophoblasts is very low. Obtaining the quantity and quality of DNA sufficient for subsequent analysis is key point in the possibility of studying the genetic material of single cells. To compare three different methods of whole genome amplification in model experiment was performed.

Materials and Methods: There were used 10 samples of artificially created mixtures. The artificial mixtures were prepared by mixing samples of peripheral venous blood of adult with cells' samples of chorionic villus with known karyotype. There was used filtration through polycarbonate filters to isolate trophoblasts from artificial mixtures. Isolated cells were fixed with paraformaldehyde during sample preparation. Detection of trophoblasts on the filters was carried out by immunocytochemical staining with monoclonal antibodies to cytokeratin 7. Isolation of single cytokeratin7-positive cells was performed by laser microdissection. WGA performed by three different **Methods:** linker adapter PCR (LA-PCR), degenerate oligonucleotide primed PCR (DOP-PCR) and multiple displacement amplification (MDA). Analysis of molecular karyotype of WGA products was performed by comparative genomic hybridization. Lengths and concentrations of WGA products and results of comparative genomic hybridization results were compared.

Results: LA-PCR showed the highest sensitivity, specificity and uniformity of amplification in comparison with other methods.

Conclusion: The results of model experiment show that not all the WGA methods used are applicable for the analysis of single trophoblast cells, isolated by filtration and fixed with paraformaldehyde in the process of sample preparation.

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P01.98B

Rare mosaicism with ring X chromosome in female patient with Turner syndrome and normal fertility: a case report

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Introduction: Turner syndrome (TS) is a chromosomal condition that affects development in females, caused by the loss of an X-chromosome or X-structural abnormalities in the X-chromosome.This condition occurs in about 1 in 2,500 newborn girls. The most frequent constitutional karyotype of TS patients is 45,X. Small percentage individuals with TS also have another cell line with 46 chromosomes due to presence of a ring chromosome X instead of normal X chromosome

Materials and Methods: We present a case of a 37-year-old TS women who had normal fertility and three documented pregnancies. Cytogenetic analysis were based on the analysis of 200 metaphases. According to standard procedures, metaphase chromosomes were obtained from phytohaemagglutinin stimulated lymphocyte cultures from peripheral blood. The chromosomes were analyzed by Giemsa banding and karyotype according to the Use of the International System for Human Cytogenetic Nomenclature.

Results: Female patient with a phenotype of TS contacted a genetic counselor after two lost pregnancy. The result of cytogenetic analysis: 45,X[188]/46,X,r(X)(p22.1q28)[12]. After a few months, the patient came pregnant and prenatal diagnosis was conducted. The third pregnancy was successfully completed by the birth of a healthy boy.

Conclusions:TS women typically experience gonadal dysfunction that results in amenorrhea and sterility. Most likely, an ovum with the ring X chromosome can be fertile and can produce a viable zygote.TS associated with an X ring chromosome, r (X), is rare and this view is our country first case report on normal pregnancy in TS with 45,X/46,X, r(X) mosaicism.

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P02 Sensory disorders (eye, ear, pain)

P02.02A

Coding and non-coding structural variants of *ABCA4*

contribute to the missing heritability in Stargardt disease, a prevalent inherited retinal disease

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Stargardt disease (STGD1) is a prevalent autosomal recessive inherited retinal disease (IRD), hallmarked by a large proportion of mono-allelic cases with one coding *ABCA4* mutation, representing an interesting cohort to elucidate missing heritability. We aimed to find the second pathogenic allele in eleven mono-allelic STGD1 patients without pathogenic coding or non-coding sequence variant after *ABCA4* locus resequencing. Targeted copy number variant (CNV) analysis using a customized platform (arrEYE) interrogating coding and non-coding regions of IRD genes, revealed four novel *ABCA4* CNVs in unrelated STGD1 patients. A 10 kb deletion spanning *ABCA4* exons 10-11 was identified in one patient. An out-of-frame deletion of exon 40-50 (19.112 bp) was identified in another patient and eliminates the 3'UTR of the gene, most likely rendering the resulting transcript unstable and prone to nonsense-mediated decay. An in-frame tandem duplication (exons 2-6) of 26 kb was identified in another patient. A non-coding intronic tandem duplication of 7 kb was identified in intron 1, occurring in *trans* with a mild mutation. We hypothesize a regulatory or splicing effect as a consequence of the duplication. All CNVs were delineated and investigated via bioinformatics analyses, pointing to a replicative-based mechanism for three out of the four CNVs and to non-homologous end joining for one CNV. These findings add to the *ABCA4* mutational spectrum, characterized by a paucity of CNVs, with eight deletions reported so far. Finally, we highlight the importance of investigating non-coding regions of *ABCA4* in mono-allelic STGD1 patients.

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P02.03B

Two novel homozygous splicing mutations in *ARL2BP* cause autosomal recessive Retinitis Pigmentosa

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Introduction: Retinitis pigmentosa is the most common inherited retinal dystrophy, affecting approximately 1 in 4,000 individuals. Mutations in *ARL2BP*, encoding ADP-ribosylation factor-like 2 binding protein, have recently been implicated as a cause of autosomal recessive retinitis pigmentosa (arRP) with 3 homozygous variants identified to date (c.101-1G>C, c.134T>G [p.Met45Arg], c.207+1G>T) in cases of Arab-Muslim, European and Moroccan origin.

Materials and Methods: Whole-genome sequencing (WGS) or whole-exome sequencing (WES) was performed in 1051 unrelated individuals recruited to the UK Inherited Retinal Disease Consortium and NIHR-BioResource Rare Diseases research studies. Sanger sequencing was used to validate the next generation sequencing data, and RT-PCR analysis was performed on RNA extracted from blood from affected individuals to test for altered splicing of *ARL2BP*.

Results: Homozygous variants in *ARL2BP* (NM_012106.3) were identified in two unrelated individuals with RP. The variants, c.207+1G>A and c.390 +5G>A, at conserved splice donor sites for intron 3 and intron 5 respectively, were predicted to alter the pre-mRNA splicing of *ARL2BP*. RT-PCR spanning the affected exon/intron boundaries showed both variants caused abnormal splicing of *ARL2BP* in samples from affected individuals compared to controls.

Conclusions: Our study identified 2 homozygous variants in *ARL2BP* as a rare cause of arRP. Further studies are required to define the underlying disease mechanism causing retinal degeneration as a result of mutations in *ARL2BP*.

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P02.04C

ATXN3 in retina: from ataxia to candidate gene for retinal dystrophies

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Introduction: An expanded CAG repeat on the *ATXN3* gene, encoding the deubiquitinating enzyme ATXN3, causes Spinocerebellar Ataxia Type 3 (SCA3), a late onset autosomal dominant neurodegenerative disorder. The physiological role of the wild-type ATXN3 protein, however, is not completely understood. Since deubiquitination seems to play a crucial role in photoreceptor development and differentiation, we aimed to define the function of ATXN3 in retinal formation combining animal and cell models.

Methods: Functional analysis in *Atxn3* murine models was performed and the retinal phenotype was studied by immunofluorescence detection, transmission electron microscopy and photoreceptor isolation for a more detailed description. A cell model of *ATXN3* knockdown was generated using RNAi in human ARPE-19 cells, which was analyzed by western-blot and immunofluorescence detection.

Results: The *Atxn3* knockout mouse retinas showed a significant elongation of the photoreceptors, mislocalization of cone-specific phototransduction proteins, with concomitant elongation of the connecting cilium -the gate through which the proteins are transported to the photoreceptor outer segment-, and diminished phagocytosis of the photoreceptor outer segments by the retinal pigmented epithelium (RPE). These results were also confirmed *in vitro* in ARPE-19 cells.

Conclusions: Our work supports the key role of ATXN3 in retinal development and maintenance, particularly in the ciliogenesis and protein trafficking in photoreceptors, thereby highlighting *ATXN3* as a good candidate gene for inherited retinal dystrophies.

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P02.05D

First audiological characteristics of autosomal dominant hearing loss caused by a novel *TBC1D24* gene alteration

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Background: To date different genetic variants in *TBC1D24* gene were causally involved in the development of neurological syndromes and profound prelingual hearing loss (HL) inherited in a recessive manner (DFNB86). In 2014 the first and so far only *TBC1D24* pathogenic variant has been linked with postlingual autosomal dominant HL (DFNA65).

Material and methods: Five-generation Polish family participated in the study. Clinical exome sequencing on the proband's DNA and family segregation analysis of the identified variants were performed. Audiological assessment included pure tone audiometry (PTA), impedance audiometry, transient evoked otoacoustic emissions (TEOAE) and auditory brainstem responses (ABRs). Vestibular system function was evaluated using ocular and cervical vestibular evoked myogenic potentials (oVEMP, cVEMP). Temporal bone computed tomography was also performed.

Results: Genetic testing revealed a novel probably pathogenic c.553G>A (p.Asp185Asn) *TBC1D24* variant, which fully segregated with HL in the studied family. Clinically, progressive HL involving mainly high frequencies was observed. No TEOAE were recorded in the study subjects and no or increased threshold of the stapedial muscle reflex was found. Function of the vestibulocochlear nerve measured by ABR was normal. No vestibular dysfunction and anatomical abnormalities of cochleovestibular system were detected.

Conclusions: Our results represent the first independent confirmation of *TBC1D24* involvement in the development of autosomal dominant HL and the first thorough clinical characteristics of *TBC1D24*-induced autosomal dominant

HL. The identified *TBC1D24* variant affects the cochlear component of the auditory system and results in a high frequency HL usually observed in the third decade of life.

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P02.06A

Integrative identification of miRNA regulatory hubs as candidate molecular discriminators of chronic otitis media pathologies

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Introduction: Chronic otitis media is followed by irreversible tissue damage and destruction of the middle ear structures. Cholesteatoma is an expanding, destructive epithelial lesion within the middle ear, commonly associated with chronic otitis media. Molecular mechanisms of chronic otitis media with cholesteatoma (COMch) and without cholesteatoma (COM) are challenging subject in research. The aim of this study was to perform transcriptome profiling of tissue from COMch and COM, and subsequent network analysis, to identify candidate miRNA regulatory hubs that can potentially serve as molecular discriminators of these two pathologies.

Materials and Methods: Transcriptome data were obtained from COMch (n = 2 patients) and COM tissue (n = 4 patients), by employing Illumina iScan microarray technology. Differentially expressed genes (DEGs) were identified using R/Bioconductor *limma* package. Functional analysis of DEGs and determination of network miRNAs by reverse mapping of target DEGs was done using miRNet software. Nonspecific miRNAs were excluded.

Results: Transcriptome analysis identified 169 DEGs. Significant biological processes involving DEGs covered epithelial cell and keratinocyte differentiation and extracellular structure organization. miRNet determined hsa-miR-8485, hsa-miR-24-3p, hsa-miR-34a-5p, hsa-miR-9-5p, hsa-miR-375, hsa-miR-145-5p, hsa-miR-21-5p as top hub miRNas with centrality degree ≥8.

Conclusions: The identified hub miRNAs should be the guide mark for future studies on mechanisms of miRNA activity in chronic otitis media pathologies. Also, hub miRNAs should be evaluated for their utilization in prevention and therapy, based on the growing potential of miRNAs to become clinically applicable biomarkers and therapeutic targets.

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P02.07B

A study of the genetics of cholesteatoma through systematic review and whole exome sequencing

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Introduction A cholesteatoma is a mass of keratinising epithelium in the middle ear. It is a rare disorder, associated with significant morbidity. Its OMIM entry (#604183) cites minimal evidence for Mendelian inheritance, but we have observed 31 multiply affected families in Norfolk; including individuals with bilateral disease, suggesting a genetic component for its aetiology.

Methods We conducted a systematic literature review (SR) to identify any published studies about the genetics of cholesteatoma and established a national biobank for subsequent whole exome sequencing (WES) studies of familial disease. We have also completed a pilot sequencing study to identify candidate variants that segregate with the disease phenotype (using NimbleGen exome capture; and the Illumina HiSeq4000 platform).

Results In our SR, we identified 8 case-series with multiply-affected families and associations with congenital malformation syndromes. DNA and clinical data have been collected from 42 participants (from 9 multiply affected Norfolk families) to date. In 2018, participants will also be recruited from 10 additional UK centres. Our pilot WES study of 16 participants from 4 families identified 95,437 variants. Variant filtering, using pedigree analysis, has identified 430 candidate genes for further filtering using the Ensembl Variant Effect Predictor.

Conclusion We have completed our SR (see PROSPERO register CRD42015023579) and established the first biobank to explore the genetics-of-cholesteatoma. A WES strategy and bioinformatics pipeline have been developed in the pilot study; and preliminary filtering has identified candidate variants that could have an impact on TGF β signalling and inflammatory processes.

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P02.08C

The relationship between defects at the middle ear bone chain and NLRP3 and OPG gene polymorphisms at chronic otitis media patients

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Introduction: The purpose of this research is to investigate the relation between osteoprotogerin, NLRP3 inflammasome gene polymorphisms and ossicular chain defects.

Materials and Methods: There were 96 participants, composed of 30 patients with type 1 tympanoplasty due to chronic otitis media between 01/06//2013 - 01/06/2016, had intact ossicular chain; 20 patients who had ossiculoplasty because of ossicular chain erosion, and 46 healthy controls. DNA was isolated from peripheral blood using Puregene Qiagen kit. OPG and NLRP3 were analyzed at real-time PCR reaction with melting curve analysis. Also inner ear swabs were used for RNA isolation and OPG and NLRP3 expression were analyzed with Real-time quantitativePCR.

Results: There were 37 males (38.5%) and 59 females (61.5%) with an age range of 15 - 70 years, The average age was 35.05 ± 14.43 years. There was no statistically significant difference between three groups according to OPG c.226A>C (p.Thr76Pro) and NLRP3 c.592G>A (p.Val198Met) polymorphisms. But when analyzed between the controls and patient group as a whole there was a significant difference of OPG c.226A>C (p.Thr76Pro) polymorphism. The expression level of OPG was higher at the tympanoplasty group compared to ossiculoplasty group

Conclusion: There was no statistically significant difference of OPG and NLRP3 gene polymorphisms among three groups. But TNFRSF11B (OPG) c.226A>C (p.Thr76Pro) polymorphism was higher at tympanoplasty and ossiculoplasty patients as a whole compared to controls. The level of OPG at inner ear swabs taken from tuba Eustachi opening at nasopharynx was increased at tympanoplasty group.

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P02.09D

***CIB2* gene in the pathogenesis of hearing loss - results of pooled DNA high throughput sequencing**

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Hearing loss (HI) is a highly heterogenic and frequent disability that affects human senses. Calcium- and integrin-binding protein 2 (*CIB2*) is one of the genes involved in HI pathogenesis. The CIB2 protein is responsible for maintaining Ca^{2+} homeostasis in cells and interacting with integrins. The aim of presented study was to establish the frequency of *CIB2*-related hearing impairment based on pooled DNA sequencing. DNA samples derived from 900 patients with HI were pooled and 180 pools were obtained (5 DNA samples/pool). The whole *CIB2* coding region was amplified in the DNA pools and sequenced on MiSeq apparatus (Illumina). Ten novel, potentially pathogenic variants were identified within the pools. DNA samples derived from pools with detected variants were separately resequenced via direct sequencing to confirm the presence of the variants as well as to assign the variants to a particular sample. Five of the primarily detected variants were confirmed in the heterozygous form, which was in line with the NGS results, the following five variants are currently being verified. Thus, our results confirmed that pool-seq is a cost and time effective approach that can be used for screening of causative HI variants in a large group of patients. This study was supported by National Science Centre grant 2011/03/D/NZ5/05592.

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P02.10A

***PAX6* sequence variants affecting splicing cause congenital aniridia**

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Introduction: Aniridia is a rare autosomal dominant panocular disorder caused by mutations in the *PAX6* gene or chromosome 11p13 rearrangements.

Materials and Methods: DNA samples for analysis were obtained from patients with congenital aniridia (110 patients from 84 unrelated families). The search for mutations in the *PAX6* gene was carried out by Sanger sequencing, MLPA and analysis of heterozygosity loss (LOH) in proband. To determine the effects of identified SNVs on PAX6 pre-mRNA splicing we used *in vitro* minigene assay.

Results: Molecular analysis of a large cohort of aniridia patients from Russia conducted earlier revealed a significant proportion of *PAX6* mutations affecting splicing (14 from 81 mutations). We focused on 8 SNVs affected slicing: 6 deep-intronic and 2 exonic. These variants were classified as variant of unknown significance (VUS), benign or likely pathogenic according to ACMG recommendations. Human Splicing Finder and IntSplice on-line tools analysis predict them to disrupt PAX6 pre-mRNA splicing. To validate this hypothesis we used a minigene system and showed that all investigated sequence variants except one affect splicing. These variants result in open reading frame shifting, premature termination codon formation following by RNA degradation by nonsense-mediated decay. Thus, investigated SNVs produce a null allele and haploinsufficiency of the *PAX6*-function. So putative mutations were reclassified as loss of function.

Conclusions: Using functional *in vitro* analysis we confirmed the pathogenicity of 7 *PAX6* mutations affecting splicing. Our results emphasized the necessity of such analysis and advanced search for *PAX6* mutations. This work is supported by RFBR grant 17-04-00475.

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P02.11B

Analysis through exome sequencing in patients with non familial primary congenital cataract

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Cataracts are one important cause of blindness around the world. Primary congenital cataract has several patterns of inheritance and can be syndromic and non-syndromic. More than 100 loci have been associated with primary congenital cataract; autosomal dominant is the principal form of inheritance. Cataract is due to opacities of the lens resulting in defects of the refractive index. This produces alterations in the lens structure resulting in light scattering with a significant concentration of high-molecular-weight protein aggregates. The aim of the present study was to analyze, through exome sequencing, a sample of Mexican patients with non-familial primary congenital cataract. Genomic DNA was extracted through conventional methods and analyzed through exome sequencing; Sanger direct sequencing was performed to confirm the exome results. Molecular analysis identified defects in the *HSF4, BFSP1, GCNT2, GJA8, BFSP1, NHS* and *MAF* genes. These alterations were not found in healthy members of the families and 100 normal controls. This study allowed to describe the genes associated with primary congenital cataract in a sample of Mexican patients and enriched the spectrum of these molecular defects associated with hereditary cataract.

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P02.12C

Evaluation of genetic variants associated with congenital stationary night blindness 2 (CSNB2)

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Introduction: CSNB2 is a non-progressive retinal disorder with clinical features that include reduced visual acuity, nystagmus and variable myopia or hypermetropia. Characteristic abnormalities are detected upon electroretinography, autofluorescence and infrared imaging. The results of these tests indicate that vision impairment in CSNB2 patients may derive from a decreased synaptic transmission between photoreceptor and second-order neurons. The Cav1.4 channel is involved in this process and CACNA1F gene encodes the pore-forming subunit α1. Since CACNA1F is located on the X chromosome, Cav1.4 channelopathies are typically affecting male patients.

Materials and Methods: We performed NGS of 32 known retinal disease genes on DNA of 3 male patients with CSNB2 and different symptoms of the disease. In two patients CACNA1F mRNA was also studied in peripheral lymphocytes.

Results: We have identified 3 unreported CACNA1F variants : in exon 4 c.425dupC, in exon 43 c.G5123C and in exon 48 c. 5800delG. The first variant causes insertion of a stop codon that could determine mRNA degradation by Nonsense Mediated Decay (NMD). However, mRNA evaluation revealed that the transcript was present. The second variant is located in a splicing site and skipping of exon 43 was demonstrated by mRNA sequencing. The third variant determinates a frameshift. In this patient mRNA was not available.

Conclusions: These results confirm that, in CSNB2 patients, variants in the same gene may be associated with different phenotypic characteristics. Moreover, they highlight the importance of studying gene expression and protein function to assess the biological significance of the variants detected.

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P02.13D

Large-scale molecular analysis of Hereditary Hearing Loss genes in Argentinean deaf patients: lookingfora needle in a haystack

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Hereditary Hearing Loss (HHL) is a common trait affecting 1 in 2000 new born children. The presence of over 100 different genes involved in HHL, lead us to go on board with Whole Exome Sequencing (WES) in order to search for the causative mutations. The main objective of this project was to diagnose Argentinean deaf families and discover novel mutations or new genes involved in pathology. We designed a flowchart to exclude all the spurious variations obtained and target for few candidates. To approach this, we filtered results from WES, and candidate variations were segregated throughout family members. Variations positively selected, were analyzed using bioinformatic predictors and tracked in public databases. Additionally, conservation studies, structure and functional domain analysis in proteins, and in-vivo studies were performed. Using this strategy we analysed 15 WES results. We identified 16 causative mutations in 12 families with syndromic and non-syndromic hearing loss (11 missense, 4 frameshift and 1 splicing site mutations). Six were novel and functional studies of some of the identified mutations, using Zebra fish models, are under way. In the remaining 3 families, variables of uncertain significance were detected (Vous). To our knowledge this is the first study using WES to diagnose deaf patients in Argentina. We show in the present study that our flowchart is advantageous and noteworthy for large-scale molecular analysis in deaf patients. These findings clearly highlight the importance of genetic studies followed by in-sílico and in-vivo validation to better understand the genetic basis of Hereditary Hearing loss.

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P02.14A

Personalized stem cell therapy to correct corneal defects due to a unique homozygous-heterozygous mosaicism of Ectrodactyly-Ectodermal dysplasia-Clefting syndrome

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Introduction: Ectrodactyly-Ectodermal dysplasia-Clefting syndrome is a rare autosomal dominant disease caused by mutations in the p63 gene. To date, approximately 40 different p63 mutations have been identified, all heterozygous. No definitive treatments are available to counteract and

resolve the progressive corneal degeneration due to a premature aging of limbal epithelial stem cells. Here, we describe a unique case of a young EEC patient, who was, surprisingly, homozygous for a novel and de novo R311K missense mutation in the p63 gene.

Material and Methods: Genetic analysis was performed in p63 gene to identify the disease-causing mutation. FISH, qRT-PCR and SNPs assay were executed to discover an additional alteration in p63. From oral biopsy, the heterozygous mutant cells were expanded performing clonal analysis.

Results: The analysis of the degree of somatic mosaicism in leukocytes and oral mucosal epithelial stem cells (OMESCs) showed that the 80% were homozygous mutant cells and 20% were heterozygous. Cytogenetic and molecular analyses excluded genomic alterations, thus suggesting a de novo mutation followed by an allelic gene conversion of the wild-type allele by de novo mutant allele as a possible mechanism to explain the homozygous condition. The R311K-p63 OMESCs were able to generate well-organized and stratified epithelia in vitro, resembling the features of healthy tissues.

Conclusion: This study supports the rationale for the development of cultured autologous oral mucosal epithelial stem cell sheets obtained by selected heterozygous R311K-p63 stem cells, as an effective and personalized therapy for this unique case of EEC syndrome, thus bypassing gene therapy approaches.

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P02.15B

Mutations in *F-Box and WD40 Domain Protein 11* (*FBXW11*) are associated with developmental eye and digit anomalies

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Defects in eye morphogenesis can lead to a spectrum of structural ocular disorders, including anophthalmia, microphthalmia and coloboma (AMC). These conditions, affecting approximately 6-13 per 100,000 individuals, are clinically and genetically heterogeneous. The number of genes known to be involved in AMC is rapidly growing, yet only approximately 25% of patients receive a genetic diagnosis. Following whole-exome sequencing of 53 individuals with developmental eye anomalies, we identified one patient with bilateral microphthalmia and polydactyly carrying a *de novo* nonsynonymous variant (NM_012300.2: c.1087C>T, p.[Arg363Trp]) in *FBXW11*. This gene codes for an F-box protein involved in the regulation of cytoplasmic levels of β-catenin and therefore plays an important role in the Wnt signalling, a fundamental growth-control pathway also implicated in eye and limb development. Screening of *FBXW11* in a further 252 patients with developmental eye anomalies identified three previously unreported 3' untranslated region (UTR) variants in three unrelated individuals. *In situ* hybridisation experiments showed *FBXW11* expression in developing human eye and brain, including the retinal neuroepithelium, telencephalon and the cerebellum. We also demonstrated that zebrafish models with reduced expression of the *FBXW11* orthologues *fbxw11a* and *fbxw11b* display smaller, misshapen and underdeveloped eyes, and abnormal pectoral fin and jaw development.

Our results support the role of *FBXW11* in eye and limb development, likely *via* modulation of the Wnt pathway, and therefore this gene represents an important candidate for human developmental disorders.

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P02.16C

Molecular diagnosis in paediatric retinal dystrophies

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Introduction: Hereditary retinal dystrophies (HRD) are a group of conditions with a great clinical and genetic heterogeneity; more than 300 responsible genes have been identified. The most prevalent HRD is retinitis pigmentosa (RP) (1: 4000).

Materials and Methods: Prospective study that includes 98 patients were evaluated in a tertiary hospital from 2010 to 2017. The clinical diagnosis was made through clinical exploration: visual acuity, biomicroscopy, fundus exploration and electrophysiological tests. According to the patient's collaboration, computerized campimetry, wide-field retinography, optical coherence tomography and microperimetry were performed. For the genetic diagnosis, a customized panel of 155 genes (IRD150) was designed by next generation sequencing and applied to children with clinical diagnosis of HRD.

Results: Identification of the causative variant was achieved in 70% of cases. In our cohort, mutations in *ABCA4* gene were the most frequent, followed by the *BEST1* gene (Best's disease) and *RPGR* (X-linked RP). 33% of molecular findings have not been previously described. Variant segregation within families was performed in order to increase evidence of pathogenicity. Adequate genetic counselling was offered in all cases. Regarding therapeutic options, 5 families with a diagnosis of ACL and mutations in *RPE65* that could be candidates for gene therapy have been identified. In 97% of cases molecular diagnosis was concordant with clinical diagnostic orientation.

Conclusion: The identification of the molecular cause in HRD is essential for diagnosis and genetic counselling to the patient and the family. In some cases, a visual prognosis and potential treatment could be provided.

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P02.17D

The improved DNA diagnostics and genetic causes of early onset hereditary hearing loss in the Czech Republic

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Hereditary hearing loss (HL) is the most common sensory deficit. About 60% of cases have genetic origin. The largest group of HL patients shows autosomal recessive inheritance. Up to 40% of HL in the Czech Republic is caused by biallelic mutations in the GJB2 gene. We have enlarged the diagnostics for the second most frequent type of HL - DFNB16. We use a simple and fast quantitative comparative fluorescent PCR and MLPA for detection of deletions or CNVs of the STRC gene. For patients who are not homozygous for the STRC deletion we use masivelly parallel sequencing (MPS) of panel of all genes already associated with autosomal recessive and X -linked types of NSHL. HaloPlex or SureSelect technology was used for library preparation.Overall 90 patients were tested by 3 consecutively updated gene panel versions (62, 67 and 70 genes were included). The causal variants on both alleles were found in 35% of patients (32 of 90). The most frequent causes of HL detected in the Czech population were biallelic probably pathogenic variants in following genes STRC, MYO15A, CDH23 and OTOG. Detection rate for each panel testing was different, namely 34%, 40% and 12.5%. In the first two panels most patients with at least one affected sibling were included. On the contrary only patients with sporadic HL were included in the last panel. Possible explanation for lower detection rate is that patients with acquired or autosomal dominant inherited HL were included in the last panel. Supported- by MH CR AZV 16-31173A

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P02.18A

HARS2 sequence variants identified in young individuals with severe sensorineural hearing impairment

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The genetics of hearing impairment (HI) is complex and highly heterogeneous, and variation in the same gene may be associated with different types of both syndromic and non-syndromic HI. Here we focus on HI, in association with the *HARS2* gene, where biallelic missense variants previously have been associated with Perrault syndrome in two studies. The published *HARS2* variants were all identified in patients, ascertained clinically because of the combination of HI and gonadal dysgenesis, the clinical hallmarks of Perrault syndrome. We have investigated the presence of *HARS2* sequence variants in 79 cases (42 females; mean age 23 ± 21.5 years) with HI. Using Next Generation Sequencing we have identified three potential pathogenic missense variants. In a family with three affected siblings (two girls age 12 and 15, and one boy age 19), we identified compound heterozygosity for the variants: p.Lys58Glu and p.Arg150Cys, and in a 6 year-old girl we identified compound heterozygosity for the variants: p.Arg150Cys and p. Arg327Gln. Supporting evidence for the functional defects

of the variants were obtained using *in silico* modeling of *HARS2* variants identified in this and previous studies, by predicting both the change in protein stability (ΔΔG) and of potential loss of function using a co-variation-based analysis of multiple *HARS2* sequence alignments. The three female patients with *HARS2* variants, compatible with autosomal recessive inheritance, are young for determining signs of ovarian dysgenesis, but will be followed carefully. The present study raises the possibility for non-syndromic HI being the only phenotypic effect of biallelic *HARS2* variation.

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P02.19B

Genetic testing of hearing loss related genes in Slovene patients with next generation sequencing

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Introduction: Hereditary hearing loss (HL) is characterized by a vast phenotypic and genetic heterogeneity. Up to date it was associated to 119 genes; 73 with non-syndromic and 46 with syndromic HL (Hereditary Hearing Loss Homepage, http://hereditaryhearingloss.org, February 2018). In Slovenians, 26.6% of congenitally deaf patients[1] and 11% of progressive HL patients[2] had biallelic *GJB2* mutations, where other genetic causes were limitedly exploited so far.

Material and Methods: 58 HL patients (12 congenital, 46 progressive HL) with negative *GJB2* and *GJB6* testing were subjected to targeted NGS with TruSightOne Sequencing Panel on the MiSeq platform followed by interpretation of variants in selected 111 HL associated genes and subsequent Sanger sequencing confirmation. Novel variants were evaluated with *in silico* prediction tools (Mutation Taster, CADD).

Results: Causative variants were detected in 33 patients in 16 genes (*COL2A1, DIAPH3, ILDR, MYH14, MYO15A, MYO6, MYO7A, PDZD7, POU4F3, SLC17A8, TBC1D24, TCOF1, TECTA, USH2A, WFS1, TMPRSS3*), most frequently in *USH2A* (19%), *TMPRSS3* (16%) and *SLC17A8* (9,7%). Six variants were not reported so far.

Conclusions: Causative variants explaining clinical presentation were detected in 28(48,3%) of *GJB2/GJB6* negative patients, a diagnostic yield comparable to other reports. In remaining patients, molecular diagnosis could not be achieved, where five patients were carrying one likely causative variant in genes associated with autosomal recessive HL. Identification of genetic cause delivers an important information for the genetic counseling and to some extend enables HL clinical prediction and verbal communication improvement with cochlear implants.

[1]Battellino *Int Adv Otol* 2011;7:372-378

[2]Battelino *J Laryngol Otol* 2012;126:763-9

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P02.20C

Evidence against *TMPRSS3/GJB2* digenic inheritance of hearing loss - practical lesson learned in the era of high throughput sequencing

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Background: Hearing loss (HL) is the most common disability of human senses and genetic factors significantly contribute to its development. For some HL genes digenic inheritance has been documented. Recently it has been proposed for *TMPRSS3* and *GJB2* recessive pathogenic variants. As the data were not convincing, we aimed to verify the hypothesis.

Material and methods: From our genetic database of HL patients with at least one *TMPRSS3* pathogenic variants (n = 42) we have selected individuals with additional pathogenic variants in the *GJB2* gene (n = 3) and recruited for the study all of the available family members. Segregation analysis of the respective *TMPRSS3* and *GJB2* pathogenic variants within the families was performed on genomic DNA by Sanger sequencing.

Results: The strategy has allowed to identify four individuals who were double heterozygous for pathogenic *TMPRSS3* and *GJB2* variants. Two individuals from two different families had *GJB2* c.35delG and *TMPRSS3* c.208delC and in two other individuals from one family *GJB2* c.35delG together with *TMPRSS3* c.1343T>C variants were found. None of these subjects has ever reported hearing problems and their hearing status was normal.

Conclusions: Access to a large genetic database of HL patients allowed us to identify as many as four individuals with concomitant pathogenic variants in *TMPRSS3* and *GJB2* genes and without HL. Our data provide evidence against *TMPRSS3*/*GJB2* digenic inheritance of HL. As high throughput sequencing is increasingly used for genetic testing, particular caution should be taken to not over-interpret the findings.

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P02.21D

Molecular Diagnostics of Hereditary Hearing Loss using Next-generation sequencing (NGS) in Estonian patients

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Introduction: Hearing loss (HL) is the most common sensory disorder worldwide. Despite this, DNA diagnostics of HL is complicated due to the heterogeneity of the disease. The most common cause for autosomal-recessive non-syndromic HL in the Caucasian population is mutations in the *GJB2* gene. We know from our previous study that the most frequent mutations found among Estonian children with early onset HL were c.35delG and p.M34T (36%) in *GJB2* gene. Still, other genes causing HL are rarely investigated in Estonian patients with HL. Study group consist of 60 probands with HL as main complaint or one of the the clinical features and who referred to geneticist during 2015-2017. In patients with isolated HL mutations in the *GJB2* gene were excluded by *GJB2* gene sequencing. Among 60 cases in 46 patients HL was the main complaint and in rest of 14 cases HL was one of the symptoms in addition to other clinical symptoms.

Methods: the NGS subpanel of genes associated with HL was performed (107 genes, Illumina TruSightOne panel) in 46 cases. For the other 14 cases with non-isolated HL only selected gene analysis or different subpanels were done.

Results: we found that the mutated genes were most commonly associated with Usher, Waardenburg and Alport syndrome. There were some mutations in genes related to non-syndromic hearing loss: *MYO15A, TECTA, MARVELD2, MYO6* and *STRC* gene.

Conclusion: The using of NGS gene subpanel analysis for HL is valuable and increased the detection of genetic etiology of HL for 33% (20 patients).

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Targeted Re-Sequencing (TRS) and high density SNP array for the molecular characterisation of Hereditary Hearing Loss (HHL)

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Introduction: HHL is characterized by a high genetic heterogeneity hampering accurate molecular diagnosis that is essential for a proper genetic counselling and therapeutic options.

Methods: 166 Italian HHL cases (46 familial and 120 sporadic) were screened with a TRS panel of 96 deafness genes, using Ion Torrent PGM™. Annotated variants were filtered according to the pattern of inheritance, frequency, and pathogenicity. In negative cases, HD-SNPs arrays were used to identify CNVs using Genome Studio software for allele detection and genotype calling followed by PennCNV analysis.

Results: Approximately 40% of cases (66/166) were positive for *GJB2* mutations, In the remaining 100 cases, TRS and SNPs array led to the characterization of approximately 30% of cases (30/100). In particular, we identified: 1) the first case of UPD involving *LOXHD1*, with the presence of both a small isodisomy segment spanning *LOXHD1* and a heterodisomy on the remaining parts of Chr18, suggesting that the UPD is the result of a non-disjunction in meiosis I, followed by trisomy rescue, 2) two large deletions in *OTOA* and *STRC*, 3) four patients (13%) with mutations in *TECTA* which is thus the second major player in the Italian population (in both autosomal recessive and dominant families), followed by *TMPRSS3* (3/100,10%), *MYO6*, *MYO7A*, *MYO15A, PDZD7*, and *ACTG1* (2/100,7% each).

Conclusions: Thanks to this approach approximately 58% (96/166) of cases were characterized confirming the large mutation's spectrum of HHL genes, as well as the

efficacy of TRS and HD-SNPs arrays in helping an accurate molecular diagnosis.

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P02.23B

Genetic analysis of Pakistani families with hereditary retinal dystrophies

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Introduction: Hereditary retinal dystrophies (RD) are a group of heterogeneous disorders caused by mutations in over 200 genes. RDs can be subdivided into different groups based on the primary degeneration of rod or cone photoreceptor cells. This study was conducted to investigate the underlying RD genes and mutation in consanguineous families from Pakistan.

Material and Methods: Families were recruited after informed consent. Peripheral blood was collected and genomic DNA was extracted according to standard procedures. Homozygosity mapping was performed using Affymetrix Gene Chip Human Mapping 250 K-NspI arrays. The data were analyzed using Homozygosity Mapper software. Primers for amplifying all exons and intron boundaries were designed with Primer3plus software followed by PCR and Sanger sequencing. Minigene splicing assay and DNA walking were performed on respective samples.

Results: Homozygosity mapping identified a novel locus in family A, one novel gene (*C8ORF37*) in family B and a large novel genomic deletion of the (*LCA5*) gene in family C.

Conclusion: Our study indicates the heterogeneous nature of retinal dystrophies in Pakistan. Although earlier studies explored number of RD families, but underlying genes are still unknown for significant proportion of Pakistani families. Theoretically the traditional screening methods were successful, however genome wide scan were further implemented to improve the detection of underlying variations in Retinal Dystrophies. Therefore the amalgamation of traditional and modern molecular techniques is required for accurate identification of mutations. It is anticipated that these findings will contribute to future genetic testing in Pakistani families to minimize the risk of recessive disorders.

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P02.24C

Variants in the *FLRT3* and *SLC35E2B* identified using whole-exome sequencing in seven high myopia families from Central Europe

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Objectives: High myopia is an eye disorder with a refractive error greater than -6 diopters (D) with both environmental and genetic factors involved. Although a number of high myopia loci have been identified, the pathogenic genes in the general population have not been determined. The aim of the study was to identify sequence variants causative for high myopia in families from Central Europe.

Methods: Seventeen individuals from seven unrelated Central European families with hereditary high myopia were assessed using whole-exome sequencing analyses. Selected variants were further evaluated using Sanger sequencing and the segregation analyses in other family members.

Results: Out of a total of 72 variants identified, two novel missense variants c.1642G>C in *FLRT3* and c.938C>T in *SLC35E2B* segregate with high myopia in the HM-78 family.

Conclusions: *FLRT3* and/or *SLC35E2B* could represent disease candidate genes and may be potentially responsible for high myopia in the HM-78 family. Our data suggest that there are different genetic backgrounds of high myopia in each multiplex family, indicating the complex genetic causes of high myopia. This study was supported by National Science Centre in Poland, (Doctoral Scholarship

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P02.25D

Exome-based RetNet panel analysis in a Belgian cohort with inherited retinal disease (IRD) expands the molecular and phenotypic spectrum of recently identified IRD genes

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Inherited retinal diseases (IRD) are a major cause of early-onset blindness, caused by mutations in >250 disease genes.

We investigated the currently known IRD genes (RetNet panel, https://sph.uth.edu/retnet) in 253 IRD probands using whole-exome sequencing (WES).

We identified (likely) causative variants in 55% of IRD probands, in frequently as well as rarely mutated RetNet genes. Specifically, two unrelated probands with simplex retinitis pigmentosa (RP) were homozygous for a novel frameshift variant in *RAX2*, in which so far only mono-allelic mutations have been described in dominant cone-rod dystrophy and macular dystrophy, suggesting a novel disease association to a known IRD gene. Secondly, *ARL3* was recently proposed as a novel disease gene for autosomal dominant RP (ADRP). Interestingly, we identified the same *ARL3* variant in an ADRP family, as well as a novel missense variant in a simplex RP patient, corroborating its involvement in ADRP. Lastly, in a proband diagnosed with non-syndromic IRD, bi-allelic truncating variants in *CEP164* were identified. Previous observations suggest a strong genotype-phenotype correlation of *CEP164*-disease, with truncating mutations causing early-onset phenotypes of dysplasia and malformation in different organs, and hypomorphic alleles causing late-onset degenerative phenotypes including IRD. Our observations therefore challenge the hypothesis that null mutations underlie the most severe end of the phenotypic spectrum.

Overall, RetNet-WES analysis revealed an underlying genetic defect in 55% of a Belgian IRD cohort, expanding their disease associations (*RAX2*), supporting their role in IRD (*ARL3*), and providing new insights into phenotypic consequences of bi-allelic loss-of-function alleles (*CEP164*).

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P02.26A

Diagnostics of inherited retinal disorders: Slovenian experience using clinical exome sequencing

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Introduction: Inherited retinal disorders are diagnostically challenging group of clinically and genetically heterogeneous diseases that may affect the entire retina or may be restricted to the macula. In addition, they may also be an ophthalmic manifestation of a systemic condition. There are approximately 261 genes associated with inherited retinal degeneration (https://sph.uth.edu/retnet/). We present our experiences using clinical exome sequencing for diagnostics of inherited retinal disorders.

Materials and Methods: 40 Slovenian patients with referral of suspected inherited retinal disorder were submitted to our institution. We performed targeted analysis of retinopathy-associated genes in the exome sequencing data. Mitochondrial sequence was also analysed based on the off-target exome reads. Filtered variants were analysed according to population frequency, characterization in ClinVar database, putative impact of the variant and predicted pathogenicity.

Results: Causative pathogenic variants were detected in 25 patients (63%). Of these, we confirmed the referral diagnosis in 23 cases, and reclassified initial diagnosis in two cases, one to mitochondriopathy and one to 16q11.2 microdeletion syndrome. In addition, we found variants of unknown clinical significance (VUS) in 6 cases and 9 cases with negative results. Altogether, we identified 19 known pathogenic and 12 novel pathogenic or likely pathogenic variants, 11 VUS and a microdeletion.

Conclusion: Our results demonstrate high diagnostic yield using clinical exome sequencing in diagnostics of inherited retinal disorders. Clinical exome sequencing should be a first-tier investigation not only because of genetic heterogeneity but also because of the potential to identify novel findings.

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P02.27B

Whole-gene panel sequencing in patients with inherited retinal dystrophies and mono-allelic variants: contribution of deep-intronic mutations and CNVs

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Introduction: Inherited Retinal Dystrophies (IRDs) are a group of rare disorders characterized by photoreceptors degeneration. Phenotypic and genetic heterogeneity hamper the diagnosis of IRDs. Although NGS technologies have shown to be helpful in this endeavor, some cases remain unsolved even after analyzing the whole exome. Remarkably, a number of patients carry only one mutated allele in a recessive gene, suggesting that screening non-coding regions could improve the diagnosis.

Materials and Methods: We performed a targeted sequencing study of 29 patients, 25 harboring mono-allelic mutations. The design comprised the entire genomic sequence of three genes (*USH2A*, *ABCA4* and *CEP290)*, the coding exons and flanking intronic bases of 76 IRDs related genes and two disease-associated intronic regions.

Results: Thirty-two mutations (8 novel) were identified in 18 probands (diagnostic rate: 62.07%). Among the variants, two were CNVs in *USH2A*, comprising one deletion (exons 22-55) and one duplication (exons 46-47) in two different patients. No deep-intronic mutations were detected.

Conclusions: Sequencing entire genes represents an intermediate strategy between exon-targeted and whole-genome sequencing, while reducing costs, time and effort. In our cohort, deep-intronic mutations may not play a significant role in the etiopathogenesis of IRDs in contrast to CNVs, which account for about 10% of the total mutations. Our approach to discover the second mutant allele in patients with mono-allelic mutations, together with our reliable CNVs detection algorithm, marks a step forward to increase diagnosis rate of IRDs.

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P02.28C

Clinical exome sequencing as a genomic approach for the diagnosis of unsolved cases of inherited retinal dystrophies

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Introduction: Genomic approaches based on the analysis of previously disease-associated genes have shown a high diagnostic efficiency in Inherited Retinal Dystrophies (IRD). However, around 40-50% of cases remain unsolved after their application.

Materials and Methods: Targeted enrichment of around 5000 clinically relevant genes (SureSelect Focused Exome, Agilent) and Illumina HiSeq 3000 sequencing, were used for the molecular diagnosis of 12 unsolved IRD cases previously analyzed by a retinal gene panel.

Results: Application of our data analysis pipeline allowed the identification of causal mutations in 4 out of the 12 cases. Three of these cases carried variants in IRD-associated genes not initially included in the panel (*FAM161A*, *MFRP* and *RP1L1*) and 1 harbored a mutation in the orf15 of *RPGR* that, although it was included in the panel, represents a highly repetitive region technically difficult to capture. Furthermore, in 2 other families, we found candidate mutations in genes not previously associated with IRD for which segregation and/or additional studies are currently pending.

Conclusions: These results allowed us to improve the efficiency of our retinal panel and suggest that most of unsolved cases could carry understudied mutations in IRD genes (deep intronic or large rearrangements) or variants in genes not yet associated with any phenotype. In this regard, it will be increasingly difficult to identify new genotype-phenotype correlations of already known genes. Therefore,

in our experience, genome sequencing would be the most appropriate approach for these cases.

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P02.29D

Iterative Sequencing and Variant Screening (ISVS) as a novel pathogenic mutations search strategy

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Autosomal recessive diseases (ARD) are typically caused by a limited number of mutations whose identification is challenged by their low prevalence. Our purpose was to develop a novel approach allowing an efficient search for mutations causing ARD and evaluation of their pathogenicity without a control group. We developed Iterative Sequencing and Variant Screening (ISVS) approach based on iterative cycles of gene sequencing and mutation screening, and *ISVS Simulator* software (http://zsibio.ii.pw.edu.pl/shiny/isvs/) for assessment of detected variants' significance. As shown by simulations, ISVS efficiently identifies and correctly classifies pathogenic mutations except for cases where the gene of interest has extremely high number of low frequency nonpathogenic variants. By applying ISVS, we found 4 known and 9 novel (p.C73Y, p.S124L, p.C194Mfs*17, c.782 + 2 T > A, c.953-5 A > G, p.L325Q, p.D334Mfs*24, p.R436G, p.M448T) *TMPRSS3* variants among deaf patients. For 3 known and 5 novel variants the disease association was supported by *ISVS Simulator* odds >90:1. Pathogenicity of 6 novel mutations has been supported by *in-silico* predictions of variants' deleteriousness. By directly comparing variant prevalence in patients and controls, disease association was demonstrated only for two variants and it was relatively weak ($P < 0.05$). Summarizing, ISVS strategy and *ISVS Simulator* are useful for detection of genetic variants causing AR diseases.

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P02.30A

Multiple differentially methylated regions specific to keratoconus overlap known keratoconus linkage loci

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Keratoconus (KTCN) is a complex degenerative eye disorder in which development both genetic and environmental or behavioral components are involved. In order to verify if DNA methylation may also play a role in KTCN etiology, reduced representation bisulfite sequencing (RRBS) of human corneas obtained from five KTCN and five non-KTCN individuals was performed. Multiple differentially methylated regions (DMRs) specific to KTCN were detected and many of them overlap previously identified KTCN linkage loci (3p14.3, 15q24.1, 20p13, 5q35.2, 13q32.3) and chromosome arms (2q, 4q, 5p, 9p, 14q, and 17q). For many of these regions candidate genes and variants were never identified and our results may allow for significant narrowing of the genomic regions of interest and reduce the list of putative KTCN genes. We also reanalyzed the previously described RNA-Seq dataset of 25 KTCN and 25 non-KTCN human corneas and found that 12 genes downregulated in KTCN (IQGAP2, SYNJ2, CYP1B1, MYO1G, WNT5A, PARVB, MGLL, CDC25B, PSG3, FHL2, CAMK1D, and THEMIS) and six upregulated genes (WNT3, RB1, AC098617.1, RPS6KA2, PELI2, and PLXNA4) overlapped or were located in the near vicinity of the identified DMRs. Particularly interesting were the DNA methylation changes in two genes encoding Wnt ligands (Wnt3 and Wnt5A), as they provide a potential explanation for the Wnt signaling pathway deregulation observed in KTCN. Supported by National Science Centre in Poland, 2012/05/E/NZ5/02127.

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P02.31B

Mutations in *TUBB4B* cause a distinctive sensorineural disease

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Leber congenital amaurosis (LCA) is a neurodegenerative disease of photoreceptor cells that causes blindness within the first year of life. It occasionally occurs in syndromic metabolic diseases and plurisystemic ciliopathies. Using exome sequencing in a multiplex family and three sporadic cases with an atypical association of LCA with early-onset hearing loss, we identified two heterozygous mutations affecting Arg391 in the β-tubulin 4B isotype-encoding gene (*TUBB4B*). Inspection of the atomic structure of the microtubule (MT) protofilament reveals that the β-tubulin Arg391 residue contributes to a binding pocket that interacts with α-tubulin contained in the longitudinally adjacent αβ-heterodimer, consistent with a role in maintaining MT stability. Functional analysis in cultured cells overexpressing FLAG-tagged wild-type or mutant TUBB4B and in patient skin-derived fibroblasts showed that the mutant TUBB4Bs were able to fold, form αβ-heterodimers and co-assemble into the endogenous MT lattice. However, the dynamics of growing MTs were consistently altered, showing that the mutations have a significant dampening impact on normal MT growth. Our findings provide a link between sensorineural disease and anomalies in MT behavior, and describe a syndromic LCA unrelated to ciliary dysfunction.

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P02.32C

Progressive shrinking of the eye and visual impairment caused by biallelic variants in the *MARK3* gene

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Developmental eye birth defects often severely reduce vision. We studied a cohort of more than 150 Pakistani consanguineous families with eye birth defects with at least two affected individuals. Families were analyzed by a combination of exome sequencing and homozygosity mapping. In one family (F105) three affected individuals were reported with progressive eye phthisis and visual impairment, and we identified a non-synonymous homozygous variant (NM_001128918.2:c.1708C>G:p.Arg570Gly) in *MARK3*. Given that MARK3 is highly conserved in flies (I: 55%; S: 67%) the fly homologue, *par1*, was knocked down in the eye during development. This resulted to a significant reduction in eye size and reduced amplitude of the electroretinograms, suggesting an impairment in visual transduction. Overexpression of the wild type Par1 protein in the developing eye caused a mild defect in eye morphology and a mild reduction in ERG amplitude. Expression of the Par1 Arg792Gly mutation, the corresponding mutation observed in the patients, induced severely reduced eye size that are rough, and cause a near complete loss of the amplitude of the ERG. Our data in flies and human indicate that the *MARK3* variants correspond to a loss of function variant in human and flies, although the precise nature of the allele remains to be determined. GeneMatcher search did not detect additional cases. MARK3 is also known to interact with Mitf, which causes the COMMAD syndrome (MIM 617306) that includes severe microphthalmia. We conclude that *MARK3* is a novel candidate for visual impairment with affected ocular anatomy.

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P02.33D

Spanish rare variants with unknown significance in candidate hearing loss genes for Meniere disease in Spain

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Introduction: The genetic architecture in Meniere's disease (MD), an inner ear disorder defined by episodic vertigo, sensorineural hearing loss (SNHL) and tinnitus, is not known. A panel of 69 hearing loss genes have been sequenced targeting rare variants in MD.

Materials and Methods: Nine hundred thirty DNA samples (890 cases and 40 controls) were pooled (each pool = 10 samples) and libraries were generated by HaloPlex PCR target enrichment system. Paired-end sequencing was performed in a Nextseq500 instrument. BWA and GATK were used for alignment and quality control. Variant calling was made through VarScan2. The estimated minor allelic frequencies (MAF) were compared with public references values in multiple populations, including Spanish variant server database.

Results: An enrichment of certain rare variants in cases was observed in genes such as *MARVELD2*. Some intronic variants with unknown significance showed a higher MAF compared with available data from Spanish population, including *SLC12A2, TRIOBP, KCNQ1* and *KCNE3* genes. Prioritizing pathogenicity prediction tools suggest that some of them should be consider as MD candidate variants. So, a novel synonymous variant in *MARVELD2* gene in 3 unrelated individuals was found and validated by Sanger sequencing.

Conclusions: Spanish population has a specific enrichment of rare variants in some hearing loss genes. The involvement of MARVELD2 variant in MD has to be investigated. The functional role of the rest of the variants in SNHL and MD remains to be established.

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Novel and ultrarare allelic variants in *DIABLO* and *SLC7A8* genes in familial Meniere's disease

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Introduction: Meniere's disease (MD), an inner ear disorder characterized by vertigo, sensorineural hearing loss and tinnitus, involves 7.5 cases in 100.000 people. MD shows familial aggregation and we have found rare variants in *FAM136A, DTNA, PRKCB* and *SEMA3D* genes in single families, showing genetic heterogeneity. We present a new family with autosomal dominant MD segregating novel variants for this condition.

Materials and Methods: A Spanish family including 3 affected women with MD, suggestive of an autosomal-dominant pattern of inheritance, was diagnosed. DNA was isolated from blood samples to perform whole-exome sequencing in the 3 cases. Single nucleotide variants (SNVs) and insertions/deletions (indels) were identified and annotated by GATK and Scalpel after quality controls. These variants were filtered by exome data from 1579 Spanish controls and the cut-off for minor allele frequency (MAF) was 0.001. Variants were prioritized according to pathogenicity by multiple bioinformatics tools.

Results: A total of 2822 rare SNPs and 779 rare indels segregated the phenotype. We identified 7 candidate variants with a MAF lower than 0.001. A novel non-synonymous SNV in *DIABLO* gene (c.C353G;p.T118R) and a non-synonymous SNV in *SLC7A8* gene (rs146946494) were the two best rated variants.

Conclusions: Familial MD shows genetic heterogeneity. This study is the basis for a forthcoming functional study to evaluate how these variants are involved in MD.

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P02.35B

The role of the gut microbiome in osteoarthritis and joint pain

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Introduction: Osteoarthritis a degenerative joint disease is predominantly thought to be due to mechanical and genetic factors. However, also chronic inflammation plays a causal role in Osteoarthritis and Osteoarthritis related joint pain. A recently proposed hypothesis suggests the involvement of the gastrointestinal (gut) microbiome in (obesity-related) knee Osteoarthritis pain, through a low grade systemic inflammation mediated by bacterial endotoxins from the gut microbiome.

Materials and Methods: Gut microbial composition was determined by 16S ribosomal RNA-sequencing (n = 1,427, Rotterdam Study population). Association analysis were done in MaAs. We used the relative abundancy of gut microbiome taxonomies, adjusted for age, sex, technical covariates, and BMI. Knee joint pain measures are based on the standardised pain questionnaires(WOMAC) pain score.

Results: We find four highly significant associations (FDR<0.05) with knee joint pain on different taxonomic levels (Class-Order-Family-Genus) leading to the bacterial genus of *Streptococcus* (FDR= 1.96E-05,). Additional adjustment for BMI did not affect the identified association Also, the relative abundancy of *Streptococcus* in the gut is significantly associated with amount of effusion (assessed by MRI), a measure for knee joint inflammation (FDR=1.1E-2, n = 373).

Conclusions: We identified a significant positive association between *Streptococcus* abundance and knee joint pain and inflammation in the knee. This association seems independent of BMI. *Streptococcus* species are known to potentially cause osteomyelitis and rheumatic fever, an inflammatory joint disease affecting the heart and articular joints, indicating that *Streptococcus* itself or released components (such as membrane vescicles), can directly target the joint.

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P02.36C

Mutation screening and tissue expression patterns implicate *SRY-box 14* (*SOX14*) in human eye and brain developmental anomalies

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Anophthalmia, microphthalmia and coloboma (AMC) are developmental eye anomalies which occur in approximately 3 in 10,000 births. They are a genetically heterogeneous group of conditions, with over 300 genes having been identified as underlying them. However, only approximately 25% of patients receive a genetic diagnosis, depending on phenotype. The most frequent genetic cause of severe AMC are alterations in *SOX2*, a member of the *SOXB* family of transcription factors which have important functions in early central nervous system development. Both SOX2 and SOX14 bind the same transcription factor binding site, acting as enhancers and repressors, respectively. Therefore, SOX14 may mediate SOX2 targeted gene transcription and so be a candidate for AMC. We screened *SOX14* in 306 individuals with developmental eye anomalies and identified four families carrying variants: a *de novo* heterozygous c.242G>T (p.Arg81Leu), a maternally inherited frameshift c.722delA, a *de novo* deletion of 7.78Mb including *SOX14*, and a paternally inherited *SOX14* duplication. However, the link between the identified variations and the ocular phenotype still remain to be demonstrated. Furthermore, *in situ* hybridisation experiments using human embryonic tissue demonstrated that *SOX14* is expressed in the eye and regions of the brain, including the hindbrain and diencephalon. Although, we developed a zebrafish model carrying CRISPR-induced mutations of *sox14*, these fish showed no alterations in eye development or gross anatomical abnormalities. We consider *SOX14* to be a likely important candidate in mammalian nervous system development and should be considered a candidate for AMC disorders.

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P02.37

Dmyopia and late-onset progressive cone dystrophy associate to LVAVA/MVAVA exon 3 interchange haplotypes of opsin genes on chromosome

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Introduction: Rare interchange haplotypes in exon 3 of the OPN1LW and OPN1MW opsin genes cause X-linked myopia, color vision defect, and cone dysfunction. The severity of the disease varies on a broad scale from non-syndromic high myopia to blue cone monochromatism. Here, we describe a new genotype-phenotype correlation attributed to rare exon 3 interchange haplotypes simultaneously present in the long- and middle-wavelength sensitive opsin genes (L- and M-opsin genes).

Materials and Methods: A multigenerational family with X-linked high myopia and cone dystrophy was investigated by clinical exome sequencing.

Results: Affected male patients had infantile onset myopia with normal visual acuity and color vision until their forties. Visual acuity decreased thereafter, along with the development of severe protan and deutan color vision defects. A mild decrease in electroretinography response of cone photoreceptors was detected in childhood, which further deteriorated in middle-aged patients. Rods were also affected, however, to a lesser extent than cones. Clinical exome sequencing revealed the LVAVA and MVAVA toxic haplotypes in the OPN1LW and OPN1MW opsin genes, respectively.

Conclusions: Here, we show that LVAVA haplotype of the OPN1LW gene and MVAVA haplotype of the OPN1MW gene cause apparently nonsyndromic high myopia in young patients but lead to progressive cone dystrophy with deuteranopia and protanopia in middle-aged patients corresponding to a previously unknown disease course. To the best of our knowledge, this is the first report on the joint effect of these toxic haplotypes in the two opsin genes on chromosome X. Supported by Ministry of National Economy, Hungary GINOP-2.3.2-15-2016-00039.

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P02.38A

A recurrent intergenic variant upstream of *PRDM13* causes autosomal dominant progressive bifocal chorioretinal atrophy in two unrelated pedigrees

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Background: Progressive bifocal chorioretinal atrophy (PBCRA) is characterised by macular (and subsequent nasal) chorioretinal atrophic lesions evident during infancy. Prior genetic linkage pinpointed the disease locus to chromosome 6q14-16.2 overlapping the North Carolina Macular Dystrophy (NCMD) locus (MCDR1), a non-progressive developmental macular dystrophy in which mutations upstream of *PRDM13* have been implicated.

Methods: Whole genome sequencing was performed to interrogate structural and single nucleotide variants in 5 PBCRA affected individuals from 2 unrelated families, including the 6q14-16.2 linked family (family 1) to gain insight into the cause of PBCRA.

Results: Seven novel variants were identified on the disease haplotype (chr6:98117898-103695199) in 3 individuals from family 1. Eleven novel variants were shared between the 2 affected individuals of family 2 at the same locus. A single variant (chr6:100046804T>C), 7.8kb upstream of the *PRDM13* gene, was identified in both families, haplotype analysis confirmed that the variant arose independently.

Discussion: We report the likely pathogenic variant in two unrelated PBCRA families and expand the non-coding variant spectrum upstream of *PRDM13*; the PBCRA variant lies 5.7kb closer to *PRDM13* than the 3 variants previously implicated in NCMD. Duplications encompassing *PRDM13* have also been implicated in NCMD.

Taken together this suggests altered spatio-temporal expression of *PRDM13* is a candidate disease mechanism in the phenotypically distinct NCMD and PBCRA. Since both disorders affect the macula at birth, exploring the functional distinctions between these variants will be key to understanding the disease mechanisms and the importance of *PRDM13* in the context of normal retinal development.

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P02.39B

Recurrence of the *SLC22A4* p.C113Y deafness-causing mutation in North Africans

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Introduction: Nonsyndromic sensorineural hearing loss (NSHL) is one of the most common congenital disorders in humans and is characterized by a high genetic heterogeneity. Recently, an homozygous missense variant (NM_003059.2:c.338G>A:p.C113Y) in the *SLC22A4* gene (*DNFB60* locus on chromosome 5), which encodes the organic cation transporter OCTN1, has been described in two Tunisian families affected by autosomal recessive NSHL.

Materials and Methods: Genome-wide linkage analysis with OmniExpressExome-8 v1.4 BeadChip array (Illumina) was performed on a large consanguineous NSHL family of Moroccan origin to highlight the genomic loci most likely to be involved in the disease. Whole-exome sequencing (WES) was then carried out on two affected siblings.

Results: Genome-wide linkage analysis on the pedigree pointed to a unique strong linkage signal peak (LOD > 3.5) in an interval of about 3Mb on chromosome 5q23.3-q31.1, encompassing the *SLC22A4* gene, delimited by markers rs11241999 and rs2237060. Moreover, WES analysis identified the presence, at the homozygous state, of the previously described p.C113Y mutation in both affected siblings. Finally, Sanger sequencing confirmed the presence of the p.C113Y variant in all 6 affected relatives, and one unaffected sibling. The entire *SLC22A4* gene was screened in additional 7 NSHL patients coming from North African countries, but no likely pathogenic variants were found.

Conclusion: This represents the first independent replication of the involvement of *SLC22A4* in autosomal recessive NSHL, highlighting the importance of this gene, and of the p.C113Y variant, at least in the North African population. This study was supported by Fondazione Cariplo (grant#2013-0825).

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P02.41D

Autosomal dominant nystagmus in a large family associated to a novel mutation in the *PAX6* gene

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Introduction: Congenital nystagmus is the most common eye movement disorder, with bilateral and involuntary oscillations of the eye, visual alteration and erroneous postures of the head. Nystagmus has been related to alterations in different genes with several patterns of inheritance. Apparently, X-linked inheritance pattern is the most common. Nystagmus can be a syndromic or non-syndromic entity. The *PAX6* gene has been associated with different eye diseases such as optic nerve/eye colobomas, aniridia, anterior dysgenesis, cataract with corneal dystrophy, foveal hypoplasia, keratitis and optic nerve hypoplasia. The *PAX6* gene is also involved in congenital nystagmus with photophobia, posterior embryoxoton and foveal hypoplasia.

Objective: In this study, we described a large Mexican family of four generations with idiopathic congenital nystagmus and no other alterations of the structures of the eye and a novel *PAX6* gene mutation.

Material and Methods: Genomic DNA was extracted from peripheral blood of 15 members of a Mexican family and 100 normal controls. They were analyzed through exome sequencing.

Results: It was detected in *PAX6* gene the mutation c.382C>T. This mutation was not found in healthy members of the family, normal controls and world databases.

Conclusion: The result include a mutation not previously reported in the literature. This mutation involves the *PAX6* gene associated to nystagmus congenital with an autosomal dominant pattern and no other clinical manifestations. This is of great relevance for the genetic diagnosis of idiopathic congenital nystagmus associated to *PAX6* gene.

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P02.42A

Functional study of a *PAX6* non-stop mutation causing autosomal dominant Retinitis Pigmentosa

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Introduction: Retinitis Pigmentosa (RP) is a group of genetically heterogeneous conditions of retinal dystrophies. PAX6 is a highly conserved transcription factor expressing in the eyes and controls the development of eyes. Null mouse mutation of *Pax6* exhibit *Small eye* (*Sey*) phenotype, similar to the heterozygous mutations of *Cad*, a gene involved in *de novo* pyrimidine biosynthesis and tightly regulated during the cell cycle. In our previous study demonstrated that PAX6 regulated transcription of *Cad*. Functional studies of *PAX6* mutations were largely unknown, our study is to investigate whether the *PAX6* non-stop mutation affects the gene expression of *CAD* as a way of functional analysis.

Materials and Methods: Structural modeling was used to predict the influence of the non-stop mutation. In vitro luciferase promoter assay and in vivo zebrafish knockdown and rescue experiments were performed to test the function of PAX6 mutation.

Results: The non-stop mutation we identified from a human RP family were predicted to have additional 36 a.a. forming an α-helical structure. We hypothesized that the mutation would lead to improper binding of PAX6 to the CAD promoter and lose transactivation ability. The luciferase promoter assay, PAX6 non-stop mutation abolished CAD promoter activity. The in vivo zebrafish morpholino knockdown with overexpressing the non-stop mutation PAX6 developed retinal abnormalities.

Conclusions: Our studies suggest that the non-stop mutation in PAX6 may reduce the expression of CAD leading to an insufficient development of the retina.

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P02.43B

Perrault-like phenotypes may account for some of the genetic and phenotypic heterogeneity within Perrault syndrome

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Perrault syndrome is a rare recessive condition characterised by sensorineural hearing loss (SNHL) and primary ovarian insufficiency (POI). Additional phenotypes, most commonly neurological, may also present. Perrault syndrome is clinically and genetically heterogeneous. Six causative genes have been identified to date, five of which function in mitochondrial homeostasis. In a number of cases no causative variants have been identified in known Perrault syndrome genes. Perrault syndrome may be difficult to clinically distinguish from overlapping phenotypes such as mitochondrial DNA depletion syndrome 7, which causes SNHL, POI and severe neurological dysfunction. We identified two cases of apparent Perrault syndrome due to variants in genes associated with other conditions. We identified a homozygous known pathogenic variant *RMND1* c.713A>G, p.(Asn238Ser) in a proband with SNHL, POI and renal acidosis. RMND1 is essential for mitochondrial translation and recessive variants in *RMND1* have been associated with renal defects, neurological phenotypes and SNHL. In a proband with SNHL, POI and mild intellectual disability we identified compound heterozygous rare variants in *XPNPEP3;* c.263A>G, p.Gln88Arg and c.1261C>G, p.His421Asp as the likely cause of the phenotype. XPNENP3 is involved in mitochondrial protein processing. Homozygous loss of function variants in *XPNENP3* have been linked to nephronophthisis with some individuals also affected by SNHL. POI has not been reported secondary to variants in *RMND1* or *XPNENP3* and reflects the later onset and sex-limited nature of this phenotype. Overlapping phenotypes such as those reported here may account for some of the genotypic and phenotypic variation seen in Perrault syndrome.

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P02.44C

12q21.33 deletion in a patient with posterior amorphous corneal dystrophy

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Posterior amorphous corneal dystrophy (PACD) (OMIM 612868) is a rare autosomal dominant disorder characterized by partial or complete posterior lamellar corneal opacification, decreased corneal thickness and flattening of the corneal curvature. Onset of the disease is in the first years of life. PACD is associated with heterozygous deletions in chromosome band 12q21.33-q22 harbouring the genes *DCN* (Decorin, OMIM 125255), *KERA* (Keratocan, OMIM 603288), *LUM* (Lumican, OMIM 600616) and *EPYC* (Epiphycan, OMIM 601657) which encode small leucine-rich proteoglycans. Only four families with deletions of this region have been published to date. We report on a 7-year-old male patient with PACD in whom an interstitial deletion in 12q21.33 was detected by array CGH. Subsequent FISH analyses in the parents revealed a balanced insertional translocation of this 12q21.33 segment into the long arm of one chromosome 13 in the mother. This family corroborates the association of 12q21.33 deletions with PACD and constitutes the first example of the involvement of a balanced chromosome aberration that predisposes to this rare disorder.

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P02.45D

First independent confirmation of the *PTPRQ* gene involvement in autosomal dominant hearing loss

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Background: Hearing loss (HL) is the most common birth defect affecting about 1-6/1000 births and the most

common disability of human senses. Genetic factors play an important role in the development of HL. The *PTPRQ* gene has been previously reported in the context of autosomal recessive HL and in 2017 for the first time in the development of autosomal dominant HL.

Material and methods: A five-generation Polish family with progressive, high frequency autosomal dominant HL was recruited for the study. Genomic DNA was isolated from peripheral blood samples or buccal swabs of available family members. Clinical exome sequencing was conducted in the proband's DNA sample. Family segregation analysis of the identified variants was performed using Sanger sequencing.

Results: Molecular genetic testing showed the presence of probably pathogenic c.6881G>A (p.Trp2294*) variant in the *PTPRQ* gene, which fully segregated with HL observed in the family. The identified variant is located in the last coding exon of the *PTPRQ* gene and introduces a premature stop codon. The c.6881G>A transition has not been reported in population databases. To date, the *PTPRQ* variant has been described in one family worldwide and is the only *PTPRQ* genetic variant causally involved in autosomal dominant HL.

Conclusions: Identification of the c.6881G>A variant provides an independent confirmation of the *PTPRQ* involvement in autosomal dominant HL, which is progressive, affects high frequencies and is usually diagnosed in the first decade of life.

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P02.46A

Using UK Biobank for common conditions of poorly characterised genetic aetiology: the example of retinal detachment

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Introduction: Rhegmatogenous retinal detachment (RRD) is a common cause of emergency ophthalmic intervention. There is some evidence for a genetic contribution to idiopathic RRD but studies have been limited. Here we evaluated the use of the UK Biobank Resource to increase insight into RRD genetic aetiology.

Material and Methods: This research has been conducted using the UK Biobank (UKBB) resources and two sets of clinically ascertained RRD cases (close to 1000 genotyped cases). A discovery genome-wide association study was carried out for retinal detachment using the largest dataset, UKBB, using BOLT-lmm which allows rapid analysis of large (N> 5000) datasets. Associated conditions, high myopia and cataract, were analysed in UKBB, to evaluate the amount of common genetic underpinning. RRD associations were replicated using the datasets of clinically ascertained cases and controls.

Results: Discovery GWAS was performed using N=3977 self reported or hospital record linked retinal detachment cases and revealed three genome-wide significant signals as well as a low but significant heritability, 24% on the liability scale. High myopia showed substantially higher heritability, with, potentially mechanistically interesting, some but not all the top signals also influencing retinal detachment. Two of the three genome-wide significant retinal detachment associations seem specific to this phenotype with no association with high myopia, cataract, nor any phenotypes from publicly available PheWAS databases. One of those hits, in the FAT3 gene, was replicated in the clinically ascertained datasets.

Conclusions. Biobank resources especially linkage to health records are a very promising complement to studies of well ascertained cases.

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P02.47B

Whole exome sequencing of a cohort of Polish patients with retinal disorders - NeuStemGen project

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The goal of the NeuStemGen project is to identify novel biomarkers, which may serve as diagnostic and prevention targets in degenerative disorders. Identification of recurrent pathogenic variants will allow to develop new therapies, such as genome editing and gene therapy, for patients with progressive, untreatable retinal dystrophies and degenerations.

The specific approach adopted, based on WES, served to identify not only pathogenic variants in genes associated retinal disorders but also in novel genes.

WES was performed for the cohort of 94 patients with clinical symptoms of retinal dystrophies while the POLGENOM genomic database of long-lived Poles was used as the control group. An in-house bioinformatic pipeline was applied to analyse SNVs and InDels. Further analysis involved Gemini, enabling an analysis of the full set of samples in search for rare pathogenic variants in the whole exome. CNVs were identified using XHMM, these results were validated using aCGH arrays (180K), showing conformity for larger structural variants.

Pathogenic variants were identified predominantly in genes already known to cause retinal degenerations (such as *ABCA4*, *USH2A*, *EYS*, *RHO*) although variants in novel genes, previously reported as single cases, were also uncovered. A gain of chr 8 was identified as a possible cause of retinal dystrophy in one patient.

The results of this analysis will support further development of the targeted retinal panel covering most pathogenic variants occurring in the Polish population and allowing for a fast, low-cost genetic analysis, preceding selection of a personalised therapy.

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P02.48C

Mutation in the intracellular chloride channel CLCC1 associated with autosomal recessive retinitis pigmentosa

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Retinitis pigmentosa (RP) is an inherited eye disease characterised by photoreceptor death and retinal degeneration, resulting in vision loss. This condition affects ~1:4000 individuals worldwide and is highly clinically and genetically heterogeneous, presenting with variable symptoms and inheritance patterns. We identified a homozygous missense alteration (c.75C>A, p.D25E) in the *CLCC1* gene, which encodes a presumptive intracellular chloride channel highly expressed in the retina, associated with autosomal recessive RP in eight consanguineous families from Pakistani and the UK. The p.D25E alteration decreased CLCC1 channel function accompanied by accumulation of mutant protein in granules within the ER lumen. In keeping with these findings, $Clcc1^{+/-}$ KO mice displayed depressed electroretinogram and photoreceptor number. Together these findings define a single founder gene mutation as a cause of RP in families of Pakistani descent, and strongly suggest that CLCC1 function is crucial for maintaining retinal integrity and function. This work was supported by National Eye Institute Grant R01EY021237-01 (SAR), National Human Genome Research Institute (NHGRI)/National Heart Lung and Blood Institute (NHLBI) to the Baylor Hopkins Center for Mendelian Genomics (UM1 HG006542, JRL), Medical Research Council UK (G1002279), the Newlife Foundation for Disabled Children (SG/15-16/12), Fight For Sight (Ref 2027), Wellcome Trust 209083/Z/17/Z.

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P02.49D

Genetic screening for Italians patients affected with Retinitis Pigmentosa

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Introduction: Retinitis Pigmentosa (RP, OMIM #268000) is a degenerative disorder affecting peripheral retina which is caused by a progressive loss of photoreceptors. The genetics of RP is highly heterogeneous, with several associated genes mainly implicated in the phototransduction cascade. RP shows autosomal dominant, autosomal recessive, X-linked and mitochondrial inheritance patterns. One of the most investigated gene associated with autosomal RP is *RHO* (3q21-q24), which encodes for Rodopsin and is essential for vision in low-light conditions. In this context, the genetics of RP was studied considering the screening of *RHO* as a first-level analysis and a panel of 24 putative genes as second-level step.

Matherial and Methods: 100 Italian RP patients were analyzed by this two steps-analysis, through direct sequencing and NGS on IonTorrent S5 (Thermo Fisher). Concerning NGS analysis, a 20X coverage was fixed as minimum depth of coverage.

Results: the first-step analysis identified a variant in the fourth exon of *RHO* gene, namely c.G760T. The variant was found in 1 patient, while the remaining 99 patients were negative for this level of analysis. These patients were therefore subjected to the second-step analysis, which reported a number of variants. At the moment, the resulting variants are under investigation and validation.

Conclusion: the present study highlights a major burden of genes other than *RHO* associated with the disease in the Italian population. Given the genetic heterogeneity of RP, it would be highly helpful and faster to perform a large-scale screening of genes compared to the slower and labor-intensive traditional approaches.

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P02.50A

Haplotypes constructed by Whole exome sequencing to map and identify a novel disease-causing *RP2* gene variant from a recessive X linked Retinities Pigmentosa family

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Introduction: Retinitis pigmentosa (RP) is a genetically heterogeneous with more than 70 RP loci currently known. A three-generation RP family exhibiting X linked recessive pattern were studied.

Materials and Methods: Linkage analysis was performed using 18 microsatellite markers. Whole exome sequencing was used for mutational analysis.

Results: Polymorphic microsatellite markers were used to map the disease interval to a 48 Mb region on the X chromosome between the markers *DXS1068* and *DXS1196*. Whole exome sequencing (WES) was performed on a selected sib-pair within the family. Total 1973 SNPs were found to be located within the critical interval. Among these SNPs, 139 were shared between the obligated carrier and the affected male offspring but not the phenotypically normal male offspring. We also utilized the WES identified polymorphic SNPs mapped within the critical disease interval to construct detailed haplotype for the sib-pair. Additional crossing-over evens were revealed on and the disease interval were mapped into two intervals: chrx: 38,911,177 -68890047; and chr X: 69,155,432-69,261,818). The causative mutation (*RP2* c.102G>A; Lys34Lys, a *RP2* splicing donor site mutation) was then identified.

Conclusion: We have combined the positional information obtained from the linkage and haplotype analysis to identify the genetic intervals harboring the disease-causing

gene, and also utilized DNA polymorphisms identified from WES as additional genetic markers to further mapped the crossing-over evens as a creative strategy for positional cloning.

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P02.51B

Improved genetic diagnostics of *RPGR* ORF15-associated retinal dystrophy

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Retinitis pigmentosa (RP) is the most common form of inherited retinal degeneration affecting around 1:3,000 individuals worldwide. Classical RP is characterized by progressive rod-cone dysfunction. Patients initially present with night blindness and tunnel vision, followed by decreased visual acuity and macular affectation. RP is clinically and genetically heterogeneous, and it can be inherited in an autosomal dominant, autosomal recessive, and X-linked manner. The majority of the X-linked RP, associated with a severe phenotype, is caused by mutations in the *RPGR* gene. All known mutations causing *RPGR*-related retinal dystrophies are found to affect the $RPGR^{ORF15}$ isoform, which contains a unique C-terminal 567-aa exon called ORF15. ORF15 is a mutational hotspot for *RPGR*-associated RP, accounting for two-thirds of all disease-causing mutations. The exon ORF15, however, includes a highly repetitive, purine-rich sequence, which generally performs poorly in next-generation sequencing (NGS)-based assays. To address the clinical importance of the *RPGR* ORF15 and the lack of high quality NGS-based diagnostics, we have developed a novel test to detect variants in the ORF15 region. This test combines NGS analysis with Illumina NovaSeq 6000 platform and Sanger sequencing, specifically optimised for this region. We have validated the test using samples with known *RPGR* ORF15 variants, and additionally tested patients with a clinical suspicion of X-linked RP, who have remained negative in previous genetic testing. We show that the test has high clinical sensitivity and specificity for detecting *RPGR* ORF15 variants, and that it improves the genetic diagnostics of inherited retinal dystrophies.

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P02.52C

SVAF retrotransposon insertion in *BBS1* gene, leading to Bardet- Biedl Syndrome

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Introduction: Active transposable elements (TE) account for over 0.02% of the human genome. One de novo insertion is believed to happen with each 10-100 live births. TEs can cause disease by inserting themselves into coding or regulatory portions of genes, facilitating duplications and deletions, among other mechanisms. Due to their repetitive nature, traditional sequencing methods often fail to detect TEs. Here we report a Bardet-Biedl syndrome (BBS) patient who is compound heterozygous for the most frequent disease-causing mutation in BBS1 and an inserted SVAF retrotransposon.

Methods and Results: Whole-genome sequencing (WGS) was performed for a female BBS patient with an identified maternally inherited mutation M390R. No other mutation was identified in BBS1 using dense microarray analysis, qPCR assays, and whole exome sequencing. An exonic TE insertion was detected in exon 13 of BBS1 in by visual inspection of the WGS read files. Following Sanger sequencing, we determined the insertion to be a paternally

inherited SVAF element of ~2 kb. We have not identified the same SVAF insertion upon screening twenty-four BBS negative patients, 2 with disease-causing mutations in BBS1. However, screening for other TE insertions are yet to be completed.

Conclusions: Disease-causing TE insertions can be missed by traditional genetic testing methods. However, they can be detected by using WGS data with specific methods, not systematically implemented. Large -scale implementation of such algorithms will allow the discovery of more disease-causing TE insertions and better assessment of the frequency of this category of mutational events.

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P02.53D

In vitro functional analysis of a novel *RP2* alleles

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Introduction: Retinitis pigmentosa (RP), a hereditary heterogeneous disease with a prevalence of 1/4000, characterized by the degeneration of photoreceptors leading to progressive loss of vision in patients. We have identified a three-generation X-linked RP family.

Materials and Methods: Eighteen X chromosome microsatellite markers were used to locate disease locus, and one of the Sibling-pairs within the family were used to performed Whole-exome sequencing.

Results: The disease locus was mapped between markers *DXS1068* and *DXS1196*. Whole-exome sequencing identified 139 possible SNPs located within the critical diseases-causing intervals. Considering that co-segregation of mutation and genes expression patterns, the *RP2_c.102G> A* point mutation might be the responsible mutation of this family's disease. It is a novel allele of RP2 gene with allele frequency <1%, and no known functional impact. At protein level the mutation appears to be no functional impact (Lys34> Lys), however, *c.102G> A* located at the last nucleotide of the splicing donor site of exon1, suggesting that this mutation might cause intron retention at the transcriptional level. A MiniGene assay was designed to validate the functional consequences of this mutation.

Conclusion: We have identified a novel splicing-site mutation of *RP2* gene from an X-Link RP family. *In vitro* functional analysis using MiniGene assay has been performed to verify the functional impact of the mutation.

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P02.54A

An integrated molecular approach to characterize the genetic bases of hearing loss in an Italian cohort

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Non-syndromic hearing loss is characterized by a vast genetic heterogeneity with more than 160 loci described in humans and 100 genes so far identified. With the aim of targeting genes strongly associated, in Caucasians, with NSHL or with SHL which onset is usually characterized by isolated deafness (i.e. Pendred and Usher syndrome), we developed an NGS targeted panel of 59 genes, to obtain an advanced efficient diagnostic tool. The Ion Torrent PGM™ platform combined with a customized bioinformatics pipeline was used for the analysis of 87 DNA samples collected from clinically highly selected Italian subjects negative for *GJB2* mutations/*GJB6* deletions. An etiological diagnosis was established in 41 of these subjects, with an overall diagnostic yield of 47%. An early molecular diagnosis of Usher syndrome was achieved in 3 unrelated children carrying mutations in *ADGRV1, CDH23* and *USH2A* genes. NGS allowed the identification of a homozygous as well as a heterozygous deletion in the *STRC* gene; the latter deletion was found in-trans with a known *STRC* pathogenic variant. The deletions were confirmed by q-PCR with primers that excluded the highly homologous *STRC* pseudogene. The novel likely causative variants identified were located in the following genes: *ACTG1, ADGRV1, CDH23, CEACAM16, COCH, COL11A2, EYA4, GJB3, KCNQ4, MYO7A, PCDH15, PTPRQ, SLC17A8, TMPRSS3*. Our targeted panel coupled with a solid bioinformatics pipeline has proved a sensitive molecular tool with a high diagnostic yield. We demonstrate the importance and efficacy of integrating the powerful NGS technology with a comprehensive data processing and a careful clinical evaluation.

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P02.55B

Stereocilin gene mutations associated with vertigo: Expansion of the DFNB16 phenotype

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Introduction: Vestibular disorders comprise a group of diseases with transient or permanent loss of vestibular function characterized by vertigo and imbalance. Isolated vestibulopathy is rare and more often associated with migraine, Ménière disease, ataxia or sensorineural hearing loss.

Materials and Methods: We examined two siblings and their first cousin with childhood onset of episodic vertigo and sensorineural hearing loss. Hearing loss and vestibular dysfunction was investigated by audiometry, SVH, cVEMP and oVEMP. DNA was analyzed using exome sequencing and SNP-array.

Results and Conclusions: Clinical investigations confirmed pathological vestibular responses in two siblings and hearing loss in all three affected individuals. The cousin had a history compatible with a vestibular disorder. DNA analysis revealed that the siblings were homozygous for a *STRC* stop variant (c.4027C>T) and their cousin was compound heterozygous for the stop variant and a 90kb deletion spanning the *STRC* gene. These results are consistent with DFNB16. *STRC*, encoding stereocilin, is expressed in the cochlea and in the vestibular organ where it ensheats the kinocilium suggesting a role for the protein in sensing balance and spatial orientation. Our findings support such a function for stereocilin in the vestibular organ and expand the phenotype associated with DFNB16.

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P02.56C

Genetic, epigenetic and environmental contributors to Age-related Macular Degeneration susceptibility

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Introduction: Susceptibility to Age-related Macular Degeneration (AMD) strictly depends on genetic, epigenetic and environmental factors. Previous results highlighted prominent differences concerning genetic contributors to AMD in Italian population compared to worldwide groups. Among genetic variables, SNPs of *CFH*, *ARMS2*, *IL-8*, *TIMP3*, *SLC16A8*, *RAD51B*, *VEGFA* and *COL8A1* were significantly associated with the risk of AMD in our cohort. Given these data, this study aimed to evaluate the contribution of genetic, epigenetic (SNPs of *miRNA-146a*, *miRNA-31*, *miRNA-23a*, *miRNA-27*, *miRNA-20a* and *miRNA-150* genes) and environment factors (age, sex, smoking, diet) to exudative AMD.

Materials and Methods: 976 exudative AMD patients and 1000 controls were subjected to an epigenotyping analysis through Real-Time PCR and direct sequencing. Biostatistical analysis was performed to calculate association and estimate the contribution of genetics, epigenetics and environment to AMD susceptibility. Gene-environment interactions were evaluated by bioinformatic tools.

Results: The SNPs rs11671784 (*miRNA-27*, G/A) and rs2910164 (*miRNA*-146a, C/G), advanced age, smoking and dietary habits were significantly associated with AMD risk ($p<0.05$). Genetic/epigenetic variants appeared to contribute to AMD susceptibility for 23% while non-genetic variants accounted for 10% of disease. Concerning gene-environment interactions, we found that AMD-associated genes may be involved in the alteration of Bruch's membrane and angiogenesis, contributing to the exacerbation of aging and environmental damages.

Conclusions: Our study provides an overview of genetic/epigenetic and non-genetic factors characterizing AMD susceptibility in Italian population. These data may be applied to develop a "population-specific precision medicine" approach able to prevent AMD or improve patients' quality of life.

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P02.58A

Discovery of new Hereditary Hearing Loss (HHL) genes by Whole Exome Sequencing (WES) and in vitro/in vivo functional studies: five years of experience

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Introduction: HHL is genetically heterogeneous and at least 40% of cases are not characterized by mutations in known genes. Thus, we applied WES followed by "in vitro" and "in vivo" functional studies for the discovery of new genes.

Methods: 14 Italian HHL families, negative for mutations in 96 deafness-genes, were analyzed by WES (Ion Proton™). Variants were filtered according to: a) pattern of inheritance, b) frequency, c) pathogenicity. Functional studies on new candidates were carried out by *in vitro/ in vivo* experiments.

Results: WES allowed the discovery of five new HHL-genes: *PSIP1* (https://doi.org/10.1038/srep18568), *TBL1Y, SPATC1L, PLS1* and *ATP2B2*. As regards *PSIP1*, a nonsense variant in a 3-generation family was identified; RNAseq and immunolabeling confirmed gene expression in mouse inner ear. For *TBL1Y*, a missense variant was detected in a large Y-linked family; functional experiments demonstrated *TBL1Y* expression in human cochlea and an early degradation of the mutated protein. For *SPATC1L*, a nonsense variant in a 3-generation family was identified; protein modeling revealed a reduced structural stability (loss of part of the C-terminus) confirmed by western blot (presence of a shorter protein isoform). Finally, for *PLS1* and *ATP2B2* (known as a *CDH23* modifier) Zebrafish KI models of the identified variants (a missense and a nonsense variant, respectively, in two dominant HHL families) are at the final stages of validation. WES data of the remaining 9 families are now under investigation.

Conclusions: Our approach, based on WES followed by functional studies, already proved to be effective for the discovery of new HHL-genes.

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P02.59B

Dominant *WFS1* mutations in a new congenital phenotype

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Introduction: Dominant phenotypes related to *WFS1* mutations were known to be less severe than the Wolfram syndrome (WS) recessive phenotype, characterized by diabetes mellitus, optic atrophy, diabetes insipidus and deafness. Dominant phenotypes included isolated low-frequency sensorineural hearing loss, optic atrophy and hearing impairment, isolated adult-onset diabetes and isolated congenital nuclear cataracts. More recently, De Franco et al. (Diabetes 2017) described a severe congenital Wolfram-like syndrome (CWLS), characterized by congenital progressive hearing loss, neonatal diabetes mellitus and cataract, due to *de novo* dominant mutations in *WFS1*.

Materials and Methods: We reported 3 unrelated cases with this new dominant phenotype, among our French cohort of 116 patients carrying at least 1 *WFS1*mutation.

Results: The dominant known p.Glu809Lys mutation was found *de novo* for 2 unrelated girls, respectively 12 and 3 years-old, who presented CWLS during the first year of life, associated with psychomotor retardation, failure to thrive, amblyopia, dysmorphic features and cerebellar hypoplasia. We also described a 20 years-old patient, who had developed with diabetes and deafness before 1 year of age and bilateral cataract diagnosed at 18 months, associated with glaucoma, amblyopia, cerebellar ataxia, short stature, hypothyroidism and hypogonadism. The clinical presentation was very suggestive of CWLS. We found a heterozygous p.His860Asp *WFS1* mutation, that was previously described at compound heterozygous state in a case of WS, but with congenital deafness and occuring De Novo, asking the question of its pathogenicity.

Conclusions: We highlight the expanding clinical spectrum of *WFS1*-related disorders with 3 case reports of severe congenital dominant phenotype.

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P02.60C

Multidisciplinary team work to get differential diagnosis of a reverse phenotype in retinal disease

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We report a case of a 23 year old woman referred to our center with a diagnosis of Leber congenital amaurosis (LCA). An accurate clinical re-evaluation detected dystrophy of retinal pigment epithelium, Franceschetti sign, exotropia and nistagmus. Clinical signs and symptoms were therefore not suggestive for LCA. A genetic counseling was then offered to this patient. We performed NGS analysis of 137 retinal dystrophy associated genes. Mutational and CNV analysis were performed. Pathogenic variants c.1666del in heterozygosis in *CEP290* gene and c.484G>A in heterozygosis in *CRB1* gene and probably pathogenic variant c.1054C>T in heterozygosis in *CACNA2D4* gene were detected. Variant c.1666del was reported in association with LCA *(Hui Wang et al., 2015)* in compound heterozygosis with a pathogenic variant in *CEP290* gene; variant c.484G>A in *CRB1* gene was reported in one family with pigmented paravenous chorioretinal atrophy (PPCRA) *(McKay et al., 2005)* dominantly inherited. The cosegregation analysis showed that proband's father carried all the three familial variants. No defined clinical symptoms of impairment visual loss were reported. Clinical and molecular findings are not always clear: therefore we recommend a multidisciplinary patient management in rare pathology. NGS data should lead to clinical revaluation, early diagnosis in mild symptomatic relatives and sometimes lead to achieve reverse phenotype. In conclusion, we requested clinical revaluation of the proband and her parents by our physicians, to gain also a clinical PPCRA diagnosis, which should justify a *CRB1* dominant disease with variable expression or incomplete penetrance.

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P03 Internal organs & endocrinology (lung, kidney, liver, gastrointestinal)

P03.01D
High diagnostic yield in well-defined cohort of ADPKD patients by conventional sequencing and NGS

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Autosomal dominant polycystic kidney disease is an inherited disease characterized by progressive cyst formation in both kidneys and renal function loss, which ultimately leads to end-stage renal failure. A well-defined clinical cohort of 339 ADPKD patients that gave informed consent and were screened for participation in a clinical trial was analysed for the presence of *PKD1* and *PKD2* mutations. The molecular analysis is relevant for the trial since disease progression is partly determined by mutation type. Mutation analysis was performed by Sanger sequencing and MLPA which resulted in a mutation detection ratio of 94%. Mutation negative patients were analysed for mutations in *GANAB*, *HNF1B* or *PKHD1* but no mutation was detected. Four mutation negative patients were sequenced with a NGS approach (Illumina HiSeq4000 platform, targets captured using custom-designed gene panel specific Agilent SureSelect^{XT}Clearseq enrichment kit). Data analysis was performed using an in house developed pipeline (stringent post-sequencing annotation pipeline based on BWA, GATK and VEP and various filtering steps in LOVD+). In 3 out of 4 patients a pathogenic mutation was detected in *PKD1* or *PKD2*. Mutations were missed by Sanger sequencing because of allelic drop-out, or a gap in the overlapping Sanger sequencing fragments. The remaining 16 patients are currently being analyzed with the same NGS approach. In conclusion, mutation analysis in a well-defined ADPKD cohort has an extremely high mutation detection rate (94%). The NGS approach is a very useful addition to standard mutation analysis techniques especially in very polymorphic regions of the genome like the *PKD1* genomic region.

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P03.02
ANGS allele drop out in a family with NPHP4 related nephronophthisis

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Here we report a family with one child died soon after birth due to bilateral renal agenesis and one terminated early pregnancy due to the same reason. The family was referred for genetic counseling and molecular-genetic testing to our lab. DNA from the affected children was not available so we perform whole exome sequencing of both parents. We analyzed the data searching for heterozygous genetic variant in the known nephronophthisis and polycystic kidney disease associated genes. However, only one heterozygous variant in exon 23 of *NPHP4* gene was detected in the mother: NM_015102.4: c.3292G>A (p.Ala1098Thr). Some authors reported few patients with only one *NPHP4* mutation so we could not exclude the possibility of incomplete penetrance of the genetic variant. We confirm the genetic finding with standard Sanger sequencing of both parents. Surprisingly, the father was also a heterozygous carrier of the same variant. We double check the finding and the NGS coverage (above 100x in both patients) and again it was missing from the NGS data of the father. It is well known that both cytosine methylation and DNA structures known as G-quadruplexes (G4s) in some region contributed to allelic dropout (ADO) in PCR based reactions and NGS library preparation. However the genetic region in proximity to our genetic variant is not GC-rich and as far as we know this region is also not methylated. Obviously ADO in NGS era is something that we still need to keep in mind and continue to investigate in our routine laboratory work.

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P03.03B

COL4A5 G624D: abundance of the Alport syndrome mutation in Russia along with Greek, Hungarian and Slovenian populations suggests it is a frequent mutation in Eastern Europe with mild phenotype

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Introduction: Alport syndrome (AS) is a familial hematuria caused by mutations in *COL4A3*, *COL4A4* and/or *COL4A5* genes which lead to defects in glomerular filtration barrier. So far, some founder mutations have been identified with many of them being region- or ethnicity-specific. *COL4A5* c.1871G>A, p.(Gly624Asp) pathogenic variant is known to be prevalent in AS patients from Slovenia (6 out of 17), Hungary (3 out of 10) and Greece and lead to late age at onset of end stage renal disease. In contrast to these populations, the mutation is considered to be rare in the US, Northern and Western Europe or Japan. Here we show that the mutation was detected in 7 AS patients out of 49 with genetically confirmed diagnosis from Russia.

Materials and Methods: The population sample contained 76 apparently unrelated pediatric patients (1 to 17 years old) from diverse range of regions in the European part of Russian Federation with confirmed or suspected diagnosis of AS according to current guidelines. NGS sequencing was performed using Ion PGM (AmpliSeq panel).

Results: We confirmed the diagnosis in 49 patients including 43 with X-linked AS (harboring *COL4A5* gene mutations) and 1 with digenic *COL4A5* and *COL4A3* inheritance. Seven of them were bearing the *COL4A5* c.1871G>A, p.(Gly624Asp) mutation which corresponds to 14% frequency in the sample. We demonstrate that the mutation is characterized by late age at onset of hematuria (>48 months) and absence of proteinuria in childhood. The research was supported by RFBR grant 18-34-00708 to L.I. S. and Minzdrav government grant №115022070016.

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P03.04C

Identification of the genetic background of Polish patients with suspected Alport Syndrome using next-generation sequencing

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Introduction: Alport syndrome (AS) is a clinically and genetically heterogeneous nephropathy associated with sensorineural hearing loss and ocular anomalies, with thin basement membrane nephropathy being at the mildest end of the disorder spectrum. In most AS cases pathogenic variants can be found in *COL4A5* (XL~80%) whereas *COL4A3* and *COL4A4* are associated with autosomal recessive (AR~15%) and dominant (AD~5%) forms.

Materials and Methods: In our study we examined a group of 36 unrelated Polish patients with suspected AS. To identify the molecular basis of the disease, we conducted next-generation sequencing (NGS) using Illumina TruSight One Sequencing Panel, which allowed simultaneous analysis of all genes encoding the subunits of type IV collagen.

Results: A genetic etiology was established in 32 patients. Overall, 31 pathogenic or likely pathogenic variants in *COL4A3*, *COL4A4* (both AR~12% and AD~12%) and *COL4A5* (XL~75%) were identified, including 10 known mutations and 21 novel variants expanding the list of *COL4A3-5* alterations. Changes were randomly distributed across all coding regions of *COL4A3-5*, with no indices of a genetic "*hot spot*", however we revealed variants repeated four times (*COL4A5*: c.1871G>A) or twice (*COL4A3*: c.2083G>A; *COL4A5*: c.2414G>T, c.3399delA) in our study group.

Conclusions: Diagnosis of patients with suspected AS was confirmed at the molecular level in ~89% cases. Our findings correspond to the data reported worldwide. NGS is efficient, reduces screening time and cost, and provides an expanding diagnostic tool to investigate the genetic backgrounds of AS, which will improve personalized diagnostics, genetic and prognostic counseling for the patients and their relatives.

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P03.05D

Genome-wide association study of sepsis-induced acute respiratory distress syndrome: discovery stage

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Introduction: The acute respiratory distress syndrome (ARDS) is a complex syndrome of severe acute hypoxemic respiratory failure. Here, we describe the results of the discovery stage for the first genome-wide association study (GWAS) of sepsis-induced ARDS.

Materials and Methods: We performed a GWAS on 672 sepsis patients admitted into intensive care units. After quality control steps and variant imputation in the Haplotype Reference Consortium data, 7.8 million variants with a minor allele frequency ≥1% were analyzed. Logistic regressions were carried out based on the Wald test, considering sex, age and the APACHE II score as covariates. GCTA-COJO was used to identify the independent loci. Gene-set enrichment analysis was assessed with EnrichR.

Results: A suggestive association ($p < 5.0e-5$) with ARDS was found for 53 independent loci (lowest $p = 2.6e-7$). Top hits were significantly enriched in genes linked to VEGF ligand-receptor interactions ($p = 3.5e-4$), and located near genes previously associated with other respiratory traits including lung function, chronic obstructive pulmonary disease, asthma, and idiopathic pulmonary fibrosis.

Conclusions: We have identified putative novel genetic variants associated with sepsis-induced ARDS. Replication

analyses are currently underway. These results will advance our understanding of ARDS pathogenesis and will likely allow identifying new therapeutic targets.

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P03.06A

Autophagy inhibition ameliorates renal cystic disease in OFD type I syndrome

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Introduction: Oral-facial-digital type I syndrome is ascribed to cilia dysfunction and characterized by abnormalities of face, oral cavity and digits and by renal cystic disease (CK). The causative gene codifies for OFD1, a centrosomal/basal body protein, necessary for primary cilia formation. Interestingly, recent data established a link between CK, primary cilia, cilioproteins and autophagy, a self-degradative process.

Results: Mass spectrometry analysis identified autophagy-related proteins among putative OFD1 interactors. In addition, we demonstrated that *OFD1*-depleted renal cells show increased autophagic flux and that OFD1 exerts a direct functional role on autophagosome biogenesis in an mTOR and cilia-independent manner. To test the physiological relevance of these findings we moved to *in vivo* studies and demonstrated enhanced autophagic flux both at precystic and cystic stages in two *Ofd1* mutants (*Ofd1*-IND and *Ofd1*;cre^{Ksp}). Moreover, we achieved renal specific inactivation in kidneys of both *Ofd1* and *Atg7*, a key player of autophagy. Histological analysis showed a significant reduction in the number and size of cysts in cre^{Ksp};*Ofd1*$^{y/fl}$;*Atg7*$^{fl/fl}$ mutants compared to cre^{Ksp};*Ofd1*$^{y/fl}$;*Atg7*$^{+/+}$ mice, as revealed by quantification of the cystic index, suggesting that increased autophagy may be strictly associated to renal cystogenesis in OFD type I syndrome.

Conclusions: Altogether our data suggest that autophagy alterations may be a common pathogenic mechanism in CK. Dissection of the molecular mechanisms underlying cyst formation in OFD type I will allow elucidating the role of autophagy in CK and could disclose new therapeutic avenues for renal cystic disease. Supported by the Polycystic Kidney Disease Foundation and the Telethon Foundation

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P03.07B

Genotype/phenotype correlations in ADPKD

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common gene kidney disorder with a worldwide prevalence of 1/400 to 1/1000. ADPKD is a genetically heterogeneous disease with two main genes PKD1 and PKD2 responsible of the disorder .Recently a new gene,GANAB has been reported to be involved in polycystic diseases(Porath et al., AJHG 2016) . We have completely analyzed the coding sequence of a large cohort of more than 4500 ADPKD patients (The genkyst cohort from the western part of France (2500 patients) and patients from different centers of France,(2000 patients).The analysis were performed first by Sanger and more recently by New Generation Sequencing (NGS). We have found an overall detection rate of 93% in our cohort,75% being mutated in PKD1 and 18% in PKD2 .We have performed a genotype/phenotype correlation (Audrezet et al., Hum Mut,2012 ;Cornec-Le Gall et al., JASN,2013) and showed that the type of mutation in PKD1, truncating mutations was 55.6 years versus non truncating mutations were associated with a 12 years delay in ESRD. We have only found 6 families with a mutation in the GANAB gene .To investigate the molecular basis of prenatal form of ADPKD we screened 42 patients with early ADPKD and we showed that additional PKD variation inherited from the unaffected parent were identified in 37.2% of patients ..These results suggest that hypomorphic mutations could explain at least a part of the clinical variability observed in ADPKD (Audrezet et al., JASN,2015)

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P03.08C

A pathogenic *PKD2* nonsense variant is highly prevalent in the Northern Portuguese population

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is a multi-organic hereditary disorder, responsible for 7-10% incident cases of end-stage renal failure (ESRF). In most populations, pathogenic variants in *PKD1* (OMIM#601313) and in *PKD2* (OMIM#173910) account respectively for 85% and 15% of ADPKD cases. Although both forms of ADPKD have similar pathogenesis, the onset of clinical manifestations and progression to ESRF occurs at young ages in patients with *PKD1* mutations, who reach ESRF on average 10-15 years earlier than those with *PKD2* mutations. The allelic heterogeneity is high for both genes, making NGS an appropriate first-tier approach to genetic diagnosis.

Methods: DNA was extracted from 40 unrelated ADPKD patients. *PKD1* and *PKD2* genes were analyzed by NGS sequencing (Ion Torrent PGM). Heterozygosity for clinically relevant variants was confirmed by Sanger sequencing.

Results: Ten of the 40 patients (25%) were heterozygous for a single-nucleotide c.181C>T transition in *PKD2* exon 1 - predicting the nonsense variant p.(Gln61Ter) - which co-segregated with a relatively mild form of ADPKD in the affected families, most of which are from a circumscribed region in the river Douro valley.

Discussion: The *PKD2* p.(Gln61Ter) variant is reported in the Mayo Clinic ADPKD Mutation Database and is not recognized as polymorphic human variation. Its high prevalence in a limited region of the north of Portugal suggests a "founder" effect. Accordingly, we have changed our genotyping approach to mildly affected ADPKD families originating from the critical geographic region, and screen first for that *PKD2* variant.

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P03.09D

Clinical and molecular aspects of the patients with Berardinelli-Seip congenital lipodystrophy types 1 and 4

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Introduction: Berardinelli-Seip congenital generalized lipodystrophy (BSCL) is a rare disorder due to homozygous mutations of *AGPAT2 (*type-1*), BSCL2 (*type-2*), CAV1 (*type-3*)* and *CAVIN1* (type-4) genes and characterized by reduced adipose tissue, muscular hypertrophy, hepatomegaly, insulin resistance and hypertriglyceridemia. Here, we report the longitudinal observation and comparison of the patients with BSCL type-1 and 4.

Materials and Methods: Six patients clinically diagnosed with BSCL from four families were enrolled. Exons and exon-intron boundaries of *AGPAT2* and *CAVIN1* were studied by Sanger sequencing method.

Results: Homozygous two known mutations (c.685G>T; c.514G>A) and a novel mutation (c.316+1G>T) were detected in *AGPAT2* in five patients from three families. They were admitted between 6 months and 11 years of age. The patients had reduced subcutaneous fat (5/5), muscular hypertrophy (5/5), enlarged hands and feet (3/5), acanthosis nigricans (2/5), hepatomegaly (3/5), hypertriglyceridemia (5/5), hyperinsulinemia (3/5) and low serum leptin level (1/5). During their follow-up period 4 to 7 years, hypertrophic cardiomyopathy and hyperinsulinemia have developed in only one patient. Last patient, a 2-year-old boy with pyloric stenosis operation history, had reduced subcutaneous fat, muscular hypertrophy, hypertrophic cardiomyopathy, and elevated CK value. Homozygous mutation in *CAVIN1* (c.259C>T) was detected. Hypertriglyceridemia and hyperinsulinemia were observed at 5 years of age.

Conclusions: As distinct from other BSCL types, pyloric stenosis and high CK values in an infant should suggest BSCL type-4. Interestingly, a previously reported patient with the same mutation of our BSCL type-4 patient had similar features including achalasia/pyloric stenosis, developmental hip dysplasia and high CK levels.

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P03.10A

A chronic obstructive pulmonary disease and interaction between single nucleotide polymorphisms of *FAM13A* gene and smoking

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Background and aims: Although smoking is a primary risk factor for chronic obstructive pulmonary disease (COPD), genetic polymorphisms within the *FAM13A* gene have been consistently reported to association with pulmonary function and/or COPD in genome-wide association studies. We aimed to investigate the effect of *FAM13A* gene variants that interact with ever smoking on COPD and emphysema risk.

Methods: Using a community-based cohort, we analyzed the association between genetic variants of *FAM13A* gene and COPD (GOLD stage >1) / emphysema (e.g., total lung volume, emphysema volume and emphysema ratio on computed tomography) risks using multivariate logistic and linear regression models (total n = 3,400). Furthermore, similar analyses were conducted after stratification by smoking status

Results: Five common variants (rs1458551, rs2609264, rs2609261, rs2609260 and rs7671167) annotated to the *FAM13A* gene were shown to have an additive effect on COPD and emphysema risk, whereas rs3756050 was only associated with a significantly higher risk for COPD (risky homozygote odds ratio (OR) = 1.49 (95% CI 1.13-1.96)). Finally, we identified significant interaction between the rs3756050 and ever smoking (P for interaction = 0.01).

Conclusions: We confirmed the previously reported association of *FAM13A* with COPD as well as emphysema risk. The genetic variant of FAM13A gene also interacted with ever smoking to affect the risk of higher COPD risk. This study was supported by the Korea Centers for Disease Control and Prevention grant (2011-E71004-00, 2012-E71005-00, 2013-E71005-00, 2014-E71003-00), the National Research Foundation of Korea grant funded by the Korea government (NRF-2016R1A2B4012155 and NRF-2017R1A6A3A11034663), and the Korea University Grant.

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P03.11B

Novel susceptibility genes candidates of chronic pancreatitis identified by whole exome sequencing

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The early onset chronic pancreatitis (CP) is often associated with mutations in a subset of CP genes. Despite of a progress in a discovery of new CP genes, the genetic cause of the disease, in the majority of the patients, remains unknown.

Aim: To identify novel susceptibility genes in early onset CP patients using whole exome sequencing (WES).

Patients and Methods: Six patients (mean age at diagnosis 10 years) with idiopathic or hereditary/familial CP with undetermined cause of the disease and their relatives were included for WES. Before, Sanger sequencing was performed to exclude the genetic causes of CP. WES data (HiSeq 2500, Illumina) were compared between index patient and affected or/and unaffected relatives. The variants were selected taking into account: *in silico* prediction (SIFT, MutTaster), minor allele frequency (MAF <0.01), gene function and expression in the pancreas, and the variant co-segregation with CP.

Results:. We detected 3 variants in known CP genes: two recurrent *CFTR* variants (p.L997F and p.R75Q) and one novel p.L100V*21 variant (introducing a STOP codon) in *CTRC*. We identified potentially pathogenic variants in 5 novel genes: *PNLIP* (p.Q323L), *GCK* (p.V101M), *TRPV6* (p.V239Sfs*53 and p.L576R), *SCNN1G* (p.I224V) and *SERPINA12* (p.G327D). The *TRPV6* and *SERPINA12* variants showed autosomal dominant inheritance. In 5/6 of the index patients, the trans-heterozygous variants in two different genes were observed.

Conclusions: Using WES approach, we identified novel variants and susceptibility genes candidates in CP. Their clinical significance needs to be elucidated by the functional and the case-control studies.

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P03.12C

The association between CEL-HYB1 allele and idiopathic/familial chronic pancreatitis in Polish pediatric patients

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Introduction: The replacement of a part of the carboxyl ester lipase gene (*CEL*) and its pseudogene (*CELP)* can create CEL-HYB1 allele which has been recently shown to elevate susceptibility to chronic pancreatitis (CP) in adults patients of European but not Asian ancestry, suggesting that it is an European CP risk factor. However, the replication studies are lacking.

Aim: To evaluate the risk associated with the *CEL-HYB1* allele in the pediatric Polish CP patients as compared to control group.

Patients and Method: We enrolled a single-center cohort of 147 unrelated CP children (mean age at diagnosis 12 years) including 64 idiopathic (ICP) or familial idiopathic (FCP) patients and 83 CP patients with various ethological risk factors (anatomical/physiological dysfunctions and/or genetics risk factors). The control group consisted of 331 ethnically matched individuals (mean age 45).The *CEL-HYB1* allele was detected using PCR, confirmed by Sanger sequencing.

Results: The *CEL-HYB1* allele is significantly over-represented in ICP/FCP patients compared to controls (9.4% vs 2.1%, P=0.0098) with OR of 4.8 (95%Cl 1.5-13.3) but not in a CP group with known ethological factors (1.2% vs 2.1%, P>0.05, OR=0.57, 95% CI (0.05-3.4). We identified two CP families with CEL-HYB1 allele and confirmed the co-segregation of the allele with the disease.

Conclusions: Our replication study show that the *CEL-HYB1* allele is a significant CP risk factor in idiopathic and familial CP pediatric patients. The screening for *CEL-HYB1* allele should be considered in CP diagnostics in European ancestry patients.

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P03.13D

Congenital adrenal hyperplasia, due to 21-hydroxylase deficiency (21-OHD), isolated or associated with Ehlers Danlos syndrome (EDS): technical and counseling problems related to molecular diagnosis

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The 21-OHD is caused by mutations in the CYP21A2 gene, that maps within a repeated module (RCCX) in a region (6p21) with a high recombination frequency. Some RCCX arrangements can complicate and significantly modify the interpretation of the genetic tests. Our goal was to obtain the correct diagnosis of complex cases by integrating: complete gene sequencing, segregation analysis of variants/SNPs, CNVs detection by MLPA. The analysis of > 1200 cases by this approach showed that: a) in the 6% of **alleles with multiple mutations** they are distributed on different genes (duplications); b) in the 33% of cases with the **Q318X mutation** there is a concomitant duplicated normal gene, resulting in a non-pathological allele; c) in 37% of cases with a **whole gene deletion**, this includes part of the contiguous TNXB gene, resulting in 21OHD potentially associated with EDS signs. In very rare cases (<1%) with particularly complex arrangements, interpretative doubts may persist. The complexity of 21OHD genetic analysis therefore requires the integration of multiple techniques and an excellent knowledge of the peculiarities of the locus. An incomplete investigation can lead to erroneous interpretations of the result with serious consequences in clinical management and genetic counseling. An accurate clinical evaluation of patients with 21OHD should always include the search for EDS signs. The approach described here has proved to be fundamental for a correct interpretation of complex arrangements and, especially in cases of pre-conceptional and prenatal tests, to improve the genetic counseling, as well as the clinical classification/management of the patients.

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P03.14A

***CYP21A2* mutations in congenital adrenal hyperplasia due to 21 hydroxylase deficiency in Turkish population**

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Congenital adrenal hyperplasia (CAH) caused by 21-hydroxylase deficiency is a common autosomal recessive disorder due to mutations in the *CYP21A2* gene. Defects in this gene lead to adrenal insufficiency, ambiguous genitalia, salt-wasting in classic form of CAH, and hyperandrogenism during childhood or early adulthood in milder form of CAH, known as nonclassic CAH (NCAH). Mutations are

mostly caused by a rearrangement during intergenic recombination between *CYP21A2* and its non-functional pseudogene *CYP21AP* with very high percentage of sequence homology. This study aimed at analyzing the frequency of 11 prevalent mutations in 183 patients. Mutations, including P30L, 8bp deletion, exon 6 cluster, L307 frameshift, R356W, R483P, I2 splice, I712N, V281L, Q318 and, P453S were analyzed by reverse-hybridization strip-based assay (CAH Strip Assay). While 62,3% of 183 patients (114/183) have wild type alleles, the revealed CAH-related variants are given in the table. Interestingly, homozygous Del8bpE3 and I2 splice mutations was found in two siblings. Segregation analysis showed that the mother was heterozygous for these compound mutations. After confirmation of paternity, we are now focussing on the possibility of uniparental disomy (UPD). Further studies for this case were planned to clarify the genetic mechanisms underlying this situation.

Table. Percentage of mutation frequencies

MUTATIONS	HETERO-ZYGOUS (%)	HOMOZ-YGOUS (%)	COMPOUND MUTATIONS			
			HETERO-ZYGOUS (%)		HOMOZ-YGOUS (%)	
P30L	1,47	–	**I2 Splice Del 8bp E3**	1,47	**Del 8bp E3 I2 Splice**	4,41
Del 8bp E3	2,94	–	**I2 Splice V281L**	1,47		
E6 Cluster	2,94	2,94	**I2 Splice Q318X**	2,94		
L307fs	–	–	**I712N Q318X**	1,47		
R356W	–	–	**V281L I2 Splice**	1,47		
R483P	–	–	**V281L Q318X**	1,47		
I2 Splice	–	–	**V281L P453S**	2,94	**Del 8bp E3 I2 Splice P30L**	1,47
I712N	2,94	–	**P30L I2 Splice Del 8bp E3**	5,88		
V281L	20,59	8,84	**L307fs Q318X R356W**	1,47		
Q318X	25,00	–	**P30L I2 Splice Del 8bp E3 V281L**	1,47		
P453S	2,94	1,47				
TOTAL	58,82	13,25	22,05	5,88		

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P03.16C
Molecular characterization of congenital hypothyroidism with "gland in situ" in Macedonia

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Introduction: Congenital hypothyroidism (CH), defined as lack of thyroid hormones at birth, is the most common neonatal endocrine disorder affecting 1 in 2000-4000 newborns worldwide. While up to 20% of CH cases are hereditary, the majority of cases are sporadic with unknown etiology. Mutations in at least 15 different genes have been associated with CH. The genetics of CH has not been studied in Macedonia previously.

Material and Methods: A multigenic sequencing of CH candidate genes including *TG, TPO*, *DUOX2, DUOXA2, SLC5A5, SLC26A4, IYD* and *TSHR* was performed in a cohort of 22 CH patients with "gland in situ" (GIS), both familial and sporadic cases, associated with permanent, transient or subclinical phenotype.

Results: Mutations were identified in 27% of patients in the *TPO, TSHR* and *DUOX2* genes. In two siblings with severe persistent CH, monoallelic c.1187_1188insGCCG mutation in *TPO* gene was detected. A child with prenatally diagnosed goiter was compound heterozygous for two mutations in *TPO* gene (c.31_50dup/ c.1313G>A). Two novel mutations were detected in *TSHR* gene in children with subclinical hypothyroidism c.1516G>A and c.692 +1_692+4delGTGA. A previously known mutation c.4637A>G in the *DUOX2* gene was detected in heterozygous state in one child with transient hypothyroidism.

Conclusion: Genetic variants were not frequently found in Macedonian CH patients, thus the etiology of CH with GIS remains elusive. Factors other than known dyshormonogenesis-associated genes or the *TSHR* have to be considered, as well as future studies with whole exome sequencing for elucidating the cause of hypothyroidism.

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P03.17D
Development of a biochip array for the rapid detection of 32 common cystic fibrosis mutations

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Introduction: Cystic Fibrosis (CF) is an autosomal recessive genetic condition which affects vital organs, mostly the lungs, by clogging them with thick, sticky mucus. Persistent

infections can lead to chronic lung problems. The disorder is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator gene (CFTR) and to date, over 2,000 activating mutations have been reported. In most populations, however, a panel of 30-35 mutations will detect over 90% of disease causing variants.

Materials and Methods: An assay was designed for detection of 32 common CF mutations and the intron 8 polypyrimidine tract variant 5,7,9 T, using genomic DNA. Following target-specific multiplex-PCR, amplicons were hybridised to a 7 x 7 array of Discrete Test Regions (DTR) on a biochip (Randox Laboratories Ltd, UK). Using the Evidence Investigator analyser. Biochips were imaged and analysed automatically. Assay run time was <3 hours. Pre-characterised samples (n = 50) containing known CF mutations were assessed and results compared against the predicate genotyping.

Results: The array was verified using 50 pre-characterised samples containing known CF mutations, as well as the intron 8 polypyrimidine tract, in order to confirm specificity of the biochip for detection of mutations in the CFTR gene. Each of the 32 targets was assessed at least once. 100% concordance was achieved.

Conclusions: This rapid screening technique enables the simultaneous analysis of 32 common mutations associated with cystic fibrosis. This will aid in the confirmation of suspected cases of CF and also in the identification of those who are carriers of a mutation.

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P03.18A

In vivo validation of phage therapy against *Pseudomonas aeruginosa* infections using zebrafish as a new model for cystic fibrosis

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To investigate the pathophysiology of cystic fibrosis (CF) several animal models have been developed including mouse, pig, and ferret; however, none of them perfectly recapitulates all human patient symptoms. On the contrary, zebrafish (*Danio rerio*) recently emerged as a powerful genetic model system to better understand CF onset and to develop new pharmacological treatments. Indeed, zebrafish embryos present only innate immune system and the zebrafish *cftr* gene is highly conserved with the human orthologue. *cftr*-loss-of-function zebrafish embryos mimic CF human defects in response to infection of *P. aeruginosa*, presenting a dampened respiratory burst response, a reduced neutrophil migration and defects in endocrine organs function.

In our previous work, we demonstrated that *P. aeruginosa* infection in mice and *Galleria mellonella* larvae could be cured by administration of phages, the natural enemies of bacteria. Phage therapy, used for decades in Eastern Europe, is gathering interest as a therapeutic alternative or a complementary treatment to antibiotics. The goal of this project is to *in vivo* validate the efficacy of phage therapy against *P. aeruginosa* infections using the CF-zebrafish animal model. Both wild-type and *cftr*-loss-of-function zebrafish embryos, were infected with *P. aeruginosa* by microinjection, followed by phage administration. The therapeutic effects of phages was evaluated, following embryo mortality, bacterial burden, neutrophil migration and immune response. In addition, we plan to combine the phage treatment with antibiotics to verify if combination of the two treatments has a positive outcome against *P. aeruginosa* infections.

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P03.19B

Diagnostic contribution of in house designed next generation sequencing panel gene test for Disorders of Sexual Development from Turkey

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Introduction: Disorders of sexual development (DSD) are defined as congenital conditions covering a wide spectrum of clinical expressions from mild hypospadias to abnormal

gonadal abnormalities causing complete sex reversal. Early diagnosis is valuable as it will impact the individual's mental well-being and overall life quality, including the advisability for genetic counseling.

Materials and Methods: We evaluated the results of 44 DSD cases for gross deletion/duplication with MLPA and for sequence alteration via in-house-designed next generation sequencing (NGS) targeted gene panel, using an Ion Torrent platform, covering all exon and exon-intron boundaries of 31 DSD associated genes. Cases with chromosomal abnormalities except one case with 47,XXY were excluded from the study group. Screening of targeted regions that were missed by AmpliSeq primer design is covered by Sanger sequencing. Segregation analysis was performed for very rare and novel missense alterations.

Results: Seven previously described and 15 possible DSD associated rare variants were identified in 10 different genes within a total of 19 cases, lead our diagnostic rate to 43%. The structural alteration in one novel coding region was simulated in three dimensional protein modeling program.

Conclusions: This study revealed new data for known and novel associated variants and acknowledged mutation frequencies in cases with DSD from Turkey. Our results underscored the critical role of early diagnosis which is valuable for proper management of gonadal tumors, expedient treatments and definitive genetic counseling for families.

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P03.20C

Association between a promoter SNP in MUC5B and idiopathic pulmonary fibrosis in the Newfoundland population

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Background: Idiopathic pulmonary fibrosis (IPF) is a late-onset, complex genetic disease characterized by inflammation and scarring of the lung parenchyma. To date, heterozygous causal variations in *TERT*, *TERC*, *SFTPC*, *SFTPA2*, that account for 2-20% of IPF, have been documented. More recently, a promoter variant (rs35705950) upstream of *MUC5B* has been shown to be associated with IPF.

Methods: All probands in this study were previously screened for variants in the four abovementioned PF genes. A TaqMan SNP Genotyping assay and a 7900HT Real-time PCR analyzer were used to genotype rs35705950 in our cohort. A case-control analysis was carried out using 110 affected individuals and 277 healthy controls from the Newfoundland population.

Results: There was a significant association between rs35705950 genotypes and IPF. The odds ratios for all individuals affected with IPF who were heterozygous and homozygous for the variant allele of this promoter polymorphism respectively were 5.4 (95% CI, 3.3 to 9.6, $P < .001$) and 12.2 (95% CI, 3.3 to 44.7, $P < .001$). Furthermore, two families displayed segregation of the variant allele with the phenotype. Using SISA, we showed that, in one of these families, the likelihood that the minor allele segregates with PF by chance is 1.56%.

Conclusions: The minor T allele of rs35705950 is associated with FPF and likely contributes to the significant proportion of FPF families without a known genetic predisposition.

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P03.21D

Improving of CRISPR/Cas9 efficacy for F508del mutation correction in cystic fibrosis

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Genome editing using CRISPR/Cas9 seems to be the most promising way for gene therapy to correct F508del mutation in *CFTR* gene in cystic fibrosis. Design of sgRNA to DNA sequence near mutation is determined by the presence of PAM for Cas9. It is possible to design only one sgRNA for F508del mutation for spCas9 - sgCFTR#1, but in HEK293T cell culture this sgRNA demonstrated low efficiency in indels formation in combination with different spCas9 (13.8%), compared to other sgRNAs to *CFTR* gene (13-18%), as well as to *GFP* gene (34.2%). qPCR data showed that sgCFTR#1 and sgGFP_1 expression levels were low and did not differ in 0 and 7 hours after transfection; but expression level of sgGFP_1 became almost 15-fold higher than sgCFTR#1 in 24 hours after transfection; 30 hours after transfection - 22-fold higher. Attempts to increase sgCFTR#1 expression by adding addition expression cassette to the plasmid, fusing sgCFTR#1 with active sgGFP_1

and using hybrid promoter were made, however, increasing of sgCFTR#1 efficacy was not received. Attempts to stabilize sgCFTR#1 by including G-quadruplexes to its sequence, shortening and addition of GG to the 5'-region also failed. Cultivation of the edited cells at lower temperature also did not lead to improved results. Further attempts to enhance sgCFTR#1 expression and its stabilization should be performed, or other Cas9 enzymes, which expand the ability to select sgRNA direct to F508del mutation can be used. The work was partially supported by Russian Science Foundation (agreement 17-75-20095 from 25/07/2017) and Russian Academy of Sciences.

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P03.22A

Diagnosis of monogenetic metabolic hepatopathies by whole-exome sequencing

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Introduction: As neonatal cholestasis is a common infant disease with more than 50 possible etiologies, fast and comprehensive diagnosis is desirable to initiate early therapy. Also for other hepatopathies, especially those with acute liver failure, rapid diagnosis is important, as they could constitute contraindication to liver transplantation or can be treated with specific therapies. We used four different whole-exome-sequencing-(WES)-based in-silico panels to analyze genes associated with different liver-related signs and symptoms.

Methods: DNA was extracted from blood samples of 66 patients (age: 0-46, median 2.50 years, 37 males). Library preparation was performed with TruSeq Nano DNA Library Preparation Kit (Illumina, San Diego, USA) or xGen Exome Research Panel (IDT, Leuven, Belgium). Libraries were sequenced on a NextSeq 500 (Illumina). Data analysis was done with the NGS pipeline megSAP (https://github.com/imgag/megSAP). Variants were filtered using GSvar (University Hospital Tübingen, Germany). Variant classification was done using Alamut Visual (Interactive Biosoftware, Rouen, France) according to standards and guidelines of American College of Medical Genetics and Genomics.

Results: In 25/66 patients we detected (likely) pathogenic variants or variants of unknown significance (VUS). The Table shows details:

Panel	signs and symptoms	genes	Number of requests	Number of patients with				
				(likely) pathogenic variants*	VUS+ pathogenic variant°	VUS*	heterozygous pathogenic variants or VUS°	no significant findings
1	Cholestasis with low gamma-glutamyltransferase	*ATP8B1, ABCB11, TJP2, AKR1D1, CYP7B1, AMACR, ABCD3, BAAT, CLDN1, HSD3B7, NR1H4, SLC25A13*	19	4	1	1	1	12
2	Cholestasis with high gamma-glutamyltransferase	*ABCB4, JAG1, NOTCH2*	13	–	–	3	–	10
3	Acute liver failure with suspected lysosomal storage disease, mitochondrial DNA depletion syndrome or Wilson disease	*MPV17, POLG, DGUOK, C10ORF2, TRMU, GFM1, BCS1L, NPC1, NPC2, ATP7B*	12	4	–	1	2	5
4	Hepatopathies/increased transaminases without primary cholestasis	*ATP7B, FBP1, G6PC, GBE1, GYS2, PHKA2, PHKB, PHKG2, PYGL, SLC37A4, SLC2A2*	22	2	–	–	7	13
			66	10	1	5	10	40

*Two variants for recessive, one for dominant traits

°for recessive traits

Conclusion: WES-based panel analysis represents a fast and comprehensive tool to diagnose hereditary hepatopathies in order to improve therapy and thus patient's prognosis. The WES-based approach further offers the possibility to analyze the remaining exome data in patients without clear diagnosis after panel analysis.

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P03.23B

Genetic linkage analysis identifies candidate modulators of reduced penetrance in heritable pulmonary arterial hypertension

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Introduction: Heritable pulmonary arterial hypertension is a rare disease inherited as an autosomal dominant disease. Mutations in the *BMPR2* gene have been detected in 75-80% of the cases. However, only about 20% of all mutation carriers develop the disease, indicating a reduced penetrance.

Material and Methods: We have studied a large family affected by pulmonary arterial hypertension and segregating with the pathogenic missense mutation p.Arg491Gln with a penetrance of ~30%. In order to identify genetic variants that may affect the penetrance in this family, we genotyped 20 mutation carriers (6 affected and 14 asymptomatic) and 12 healthy individuals with the Illumina Infinium CoreExome-24 BeadChip.

Results: Genetic linkage analysis showed two candidate regions in chromosome 2 (q24.2-q31.1 and q33.1-q33.3) to host the modifier and confer susceptibility to the disease among *BMPR2* mutation carriers. The modifier is predicted to be common in the population and to be inherited in an autosomal recessive mode. The first region is located 30 Mb upstream from *BMPR2*, while the second region overlaps the *BMPR2* locus itself.

Conclusions: We found a significant enrichment of HPAH-related traits by a regulatory region within q24.2-q31.1, located upstream from the *FIGN* gene. Full genome sequencing and functional assays will be required to validate the linkage regions, identify the actual variant modulating the penetrance and unravel the molecular mechanism that triggers the disease in the family under study.

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P03.24C

Search for genetic risk factors in Hirschsprung's disease associated enterocolitis by Whole Exome Sequencing

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Introduction: Hirschsprung's disease (HSCR) is a congenital gut malformation. The most serious and life-threatening complication is enterocolitis (HAEC), which occurs in one third of the patients. The evident susceptibility to HAEC in HSCR patients suggests a genetic background that can lead to abnormal inflammatory response.

Materials and Methods: To search for genetic factors predisposing to HAEC, we have performed an exome next generation sequencing (NGS) on 24 HSCR patients, 12 with enterocolitis episodes (HAEC) and 12 without (HSCR-only). Patients were selected based on Italian ancestry and absence of additional anomalies. The exomes were sequenced with Illumina at a 50X coverage and variants were filtered based on depth >= 10 and quality >= 10, obtaining 77396 variants. These were further selected based on allele frequency in databases and impact on the protein, and on higher frequency in HAEC than in HSCR-only patients.

Results: We have thus identified 86 variants in 90 genes, which were ranked based on their frequency in the general population, predicted effect, association p-value, and recurrence in the samples. We also explored the gene

pathways, biological role, and PubMed citations, particularly in regard to immune and inflammation processes. We are currently validating the five top variants and replicating the results on a larger panel of 25 HAEC and 45 HSCR-only patients.

Conclusions: We have identified a few very promising candidate genes. The most promising variant/s will undergo functional tests to confirm its/their role in HAEC development.

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P03.25D

Whole exome sequencing to detect the cause of early onset nephrocalcinosis

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Idiopathic infantile hypercalcemia (IIH) is characterized by severe hypercalcemia with failure to thrive, vomiting, dehydration, and nephrocalcinosis (NC). NC has not a well understood genesis. Monogenic causes were restricted to rare genetic syndromes/tubulopathies.

We examined a 24 month old child for advanced bilateral medullary nephrocalcinosis (accidentally detected at 15 months), failure to thrive, mild hypercalcemia/hypercalciuria, moderate hypophosphatemia without proximal tubulopathy, renal failure and skeletal dysplasia. He's the firstborn of healthy consanguineous parents and the mother was pregnant; a maternal uncle has had recurrent nephrolitiasis since the age of 22 years old. Whole exome sequencing (WES) was performed in order to clarify the molecular diagnosis and to offer a prenatal test.

WES revealed a novel homozygous *SLC34A1* mutation linked to IIH and Fanconi renotubular syndrome 2, and a monoallelic *EHHADH* mutation, inherited only from the mother that presented bilaterally kidney stones and urinary calculi history, associated with Fanconi renotubular syndrome 3. The same genetic background was identified also in fetal DNA extracted from amniotic fluid.

Treatment of IIH in early age is problematic as vitamin D therapy often exacerbates hypercalcemia. Considering the possibility to check an affected subject from the first month of life since the mother decided to continue the pregnancy, our data could give new insights about the temporal expression profile of *SLC34A1* in the kidney and an explanation to the development of hypercalcemia in childhood, where intestinal absorption and renal handling of calcium and phosphate likely differ from older patients with other forms of IIH.

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P03.26A

The application of targeted sequencing and whole exome analysis to identify disease-causing variants in familial pulmonary fibrosis

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Introduction: Idiopathic Pulmonary Fibrosis (IPF) is one of the most common forms of interstitial pneumonia and is irreversible. It has a late age of onset, mostly between 55-75 years (median 66 years) and average survival rate of 3 years post diagnosis. Using next-generation sequencing (NGS) of whole exome (WES) and a targeted respiratory gene panel (Respigene) on Finnish and UK families with familial PF (FPF), we identified many new potentially pathogenic variants causative of FPF.

Materials and Methods: WES and targeted analysis of 42 genes previously associated with interstitial lung disease (ILD) was performed on 25 individuals (21 affected and 4 unaffected) from families with severe, early-onset FPF.

Variant assessment was performed according to ACMG guidelines, using an in-house bioinformatics pipeline for classification of SNVs and CNVs, configured to rare respiratory conditions.

Results: Twelve of 25 individuals were found to have variants in ACMG classes 3-5 (VUS to pathogenic), in genes previously associated with FPF and which segregated in families where available. Seven patients were found to have potentially pathogenic variants in TERT, TERC and RTEL1, genes all previously associated with telomerase and telomere integrity. 13/21 (61.9%) patients were positive for the MUC5B SNP, rs35705950, as opposed to 1/4 unaffected individuals. No CNVs were found in any genes related to ILD.

Conclusion: These findings confirm that genes involved in telomere function play a major role in familial pulmonary fibrosis. These findings have potential implications for family members and raise the possibility of predictive testing and early therapeutic intervention in FPF.

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P03.27B

A metabolic biomarker for idiopathic pulmonary fibrosis: a two sample Mendelian randomization study and a case-control study

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a lethal disease without current effective treatments. There is, therefore, an urgent need to find and validate biomarkers for diagnosis, prognosis, as well as potential drug targets. We propose to combine genomics and metabolomics to meet these objectives.

Methods and Results: To identify potential metabolites associated with IPF, we used an IPF GWAS that identified novel single nucleotide polymorphisms (SNP) associated with isovaleryl dehydrogenase (*IVD*), whose inhibition is known to increase the metabolite isovalerylcarnitine (IVC). Concurrently, we collaborated in a metabolite GWAS in healthy subjects that found the same IPF-associated SNP to be associated with IVC blood levels. Through 2-sample Mendelian randomization, we observed that genetically decreased IVC levels were strongly associated with increased risk of IPF ($p< 2.9x10-10$). The non-coding allele that decreased IVC levels increased *IVD* expression ($p< 3.4x10-12$). Thus, we hypothesized that low IVC levels are associated with higher risk of IPF. To test our hypothesis, we ran a case-control pilot study and found that IVC levels are decreased in IPF cases relative to controls ($P = 2x10-3$). Therefore, these data strongly suggest the IVC metabolic pathway could play a role in IPF etiology.

We are currently confirming our results in a larger case-control study.

Conclusion: IPF presents seriously unmet clinical needs. Building on strong preliminary data, we propose that IVC levels are associated with higher risk of IPF. IVC represents a clinically relevant biomarker and its enzyme, IVD, could represent an entirely novel IPF drug target.

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P03.28C

Interallelic interactions mediated by the oligomerization of podocin

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NPHS2, the major gene of steroid-resistant nephrotic syndrome, encodes podocin, a membrane-anchored component of the slit diaphragm. We formerly showed that its R229Q variant is pathogenic only when trans-associated to specific 3' missense mutations secondary to an altered C-terminal dimerization. We aimed to determine the membrane targeting and the oligomerization of podocin in function of its C-terminal integrity. Podocin localization was studied in podocytes co-transfected with GFP- and HA-tagged

podocin variants. Oligomerization was assessed by measuring the FRET efficiency between maleimide-stained podocin variants and by size exclusion chromatography. We found the oligomerization to occur exclusively through the C-terminal tail (residues 283-382): principally through the 283-313 (H1) and the 332-348 residues. The interallelic interactions are mediated by oligomerization: while the monomer-forming R286Tfs*17 podocin remained membranous irrespective of the coexpressed podocin variant, podocin variants with an intact H1 significantly influenced each other's localization (r^2=0.68, $P = 9.2x10^{-32}$). The dominant negative effect resulting in intracellular retention of the pathogenic F344Lfs*4-R229Q heterooligomer occured in parallel with a reduction in the FRET efficiency, suggesting a conformational rearrangement. In contrast, oligomerization with membranous podocin variants prevented the endocytosis of F344Lfs*4 or F344* podocin mutants induced by C-terminal truncation. In conclusion, oligomerization of podocin can mediate both a dominant negative effect and interallelic complementation. Interallelic interactions of 3' *NPHS2* mutations are not restricted to the R229Q variant and have to be considered in compound heterozygous individuals. Supported by MTA-SE Lendulet Research Grant (LP2015-11/2015), NKFIA/OTKA K109718, K116305, KH125566, MedinProt Synergy grant, (ANR-10-IAHU-01), EURenOmics (2012-305608), ANR (GenPod project ANR-12-BSV1-0033.01).

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P03.29D

Biallelic Mutations in *LRRC56*, encoding a protein associated with intraflagellar transport, causes defects in mucociliary clearance and laterality

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Defects in motile cilia result in conditions characterised by impaired pulmonary mucus clearance, susceptibility to chronic recurrent respiratory infections, male infertility and laterality defects. Here we report that biallelic variants in *LRRC56* (also known as ODA8), result in a phenotype comprising laterality defects and chronic pulmonary infections. Investigation of cultured patient epithelial cells revealed severely dyskinetic cilia, but no structural abnormalities on transmission electron microscopy. In human cells, we show that LRRC56 interacts with the intraflagellar transport (IFT) protein IFT88.

In the model organism *Trypanosoma brucei*, *we* show LRRC56 is recruited during later phases of flagellar assembly before being removed during flagellum maturation, after cell division. We disrupted anterograde or retrograde IFT in T Brucei by use of RNAi. This showed that LRRC56 acts as an IFT cargo, but only at later stages of flagellar construction.

LRRC56 localisation was unchanged in $DNAI1^{RNAi}$ and $ODA7^{RNAi}$ mutants that are defective in their dynein arm constitution or cytoplasmic preassembly, respectively. Thus LRRC56 does not require the presence of dynein arms to be associated with flagella.

In *T. brucei* carrying LRRC56 null mutations, or a mutation (p.Leu259Pro) corresponding p.Leu140Pro variant seen in one of the affected families, we observed abnormal ciliary beat patterns and an absence of outer dynein arms restricted to the distal portion of the axoneme. These findings confirm that deleterious variants in *LRRC56* result in human disease, and suggest this protein has a likely role in dynein transport during cilia assembly that is evolutionarily important for cilia motility.

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P03.30A

Metformin induced changes in gut microbiome composition in healthy individuals and newly-diagnosed type 2 diabetes patients

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Metformin is a biguanide class agent widely used as a first-line treatment for type 2 diabetes (T2D). Despite its advantages, metformin has variable therapeutic effects, contraindications, and side effects. Previous findings have led to the hypothesis that both the beneficial and adverse effects of metformin are partially explained by its interaction with the gut microbiome, yet details of these mechanisms remain obscure.

The study was conducted to investigate the effects of metformin treatment on the composition of human gut microbiome. The microbial DNA was extracted from:

(1.) Stool samples obtained from 18 healthy nondiabetic individuals at three time points during metformin treatment: M0 - before metformin treatment, M24h - 24 hours after the first metformin dose, and M7d - after a week-long metformin administration;

(2.) Stool samples acquired from 11 newly-diagnosed T2D patients at two concordant time points - M0 and M7d.

Probable changes in the gut microbiome induced by metformin intake were assessed employing massive parallel sequencing of the *16S rRNA* gene V3 region.

A week-long metformin treatment rapidly and significantly decreased alpha diversity of gut microbiome among both groups. Concurrently, significant changes in several taxonomic groups were observed, including an increase in abundance of opportunistic pathogens representing *Escherichia-Shigella* genus, and decrease in possibly harming family *Peptostreptococcaceae*. Together these findings support the role of metformin in the modulation of the gut microbiome composition and the hypothesis that there may be some specific interactions further linked with efficacy and side effects of metformin.

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P03.32C
Targeted-NGS panel for inherited nephropathies: an example of clinical utility in a tube

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Introduction: Kidney diseases can be caused by a wide spectrum of underlying conditions and, in practice, the exact cause of a patient's renal disease often remains unknown. Knowing the precise genetic defect is important in terms of its diagnostic, prognostic value and appropriate genetic counselling and is likely to become more crucial for directing specific therapy. NGS has opened up a new field allowing the identification of previously undiagnosed hereditary nephropathies. This study aims to investigate the etiology of genetic kidney diseases in the clinical routine of our hospital´s patient population.

Materials and Methods: The Nefroseq® panel V2.0 is a targeted NGS panel containing 350 genes involved in inherited nephropathies, optimizing libraries from Kappa, Target enrichment by SeqCap EZ system (RocheNimblegen), and sequencing on a NextSeq500 platform (Illumina).

Results: Regarding the analysis, it is done differently depending on the patient: restricted to the proband and known genes in patients with a specific phenotype, or open to a trio (including parents) when clinicians suggest an unspecific phenotype. Diagnostic yields are around 51-54%. Remarking is the genetic analysis of the *PKD1* (responsible for most ADPKD cases), a diagnostic challenge: the 5′-region of the gene overlapped with a pseudogene. Our approach "in a tube" validated by Long-Rage PCR and Sanger sequencing yielded around 80% and it is cost-effective.

Conclusions: We share our management of the inherited nephropathies cases under a model pointed out to precision medicine, which includes a multidisciplinary committee for discussing the cases included in this customized NGS panel.

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P03.33D
Genetic causes of early onset obesity are frequently identified in a tertiary pediatric obesity cohort

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Obesity is predominantly considered a multifactorial disorder. In unselected patient cohorts, an underlying genetic diagnosis can be established in only a minority of cases.

They typically present with early-onset, severe obesity. Establishing a genetic diagnosis can lead to personalized treatment, reduce stigma and support reproductive decision-making. This study provides an overview of obesity-associated mutations and copy number variations (CNV's) identified in this selected population.

In 174 obese children, referred to the pediatric obesity center Centrum Gezond Gewicht between 2012 and 2017, diagnostic sequencing of 52 obesity-associated genes, CNV detection by SNP-microarray analysis and, on clinical suspicion, specific additional diagnostics were performed to identify genetic causes of obesity.

In 28 patients (16.1%), an underlying genetic cause was identified (table 1). In an additional 22 patients (12.6%), a novel CNV or sequence variant of unknown clinical significance (VUS) was shown in obesity-associated genes, for which the role in the phenotype has yet to be confirmed.

A definitive obesity-associated genetic diagnosis was made in 16.1% of our patients with early-onset obesity. This may increase, if follow-up studies in patients with VUS confirm a causal role for their variants. This diagnostic yield is relatively high compared to similar studies and shows that genetic testing can be highly relevant in selected obese patients, especially when personalized treatment becomes available.

Table 1. Underlying genetic causes identified in our pediatric obesity patient cohort

Genetic disorder	No. of patients	Characteristics
Melanocortin-4 receptor, dominantly inherited obesity	5	Heterozygous *MC4R* mutation
Melanocortin-4 receptor deficiency	2	Homozygous or compound heterozygous *MC4R* mutation
Leptin receptor deficiency	5	Homozygous or compound heterozygous *LEPR* mutation
16p11.2 deletion syndrome	3	Typical 16p11.2 deletion
Melanocortin-2 receptor accessory protein 2 associated obesity	2	Heterozygous *MRAP2* mutation in 2 sibs
Pseudohypoparathyroidism type 1A	2	Heterozygous *GNAS* mutation
Central hypothyroidism	1	Heterozygous *IGSF1* mutation
Cohen syndrome	1	Homozygous *VPS13B* mutation
Mental retardation, autosomal dominant 39	1	Heterozygous *MYT1L* mutation
Maternal UPD 14	1	mUPD 14
Proprotein convertase-1 associated obesity	1	Heterozygous *PCSK1* mutation
Proopiomelanocortin associated obesity	1	Deletion 2p, including *POMC* gene
Pseudohypoparathyroidism type 1B	1	Heterozygous *STX16* mutation
Single-minded 1 associated obesity	1	Deletion 6q16.3, including *SIM1* gene
Spastic paraplegia type 11	1	Compound heterozygous *SPG11* mutation

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P03.35B

Isolated oesophageal atresia associated with rare structural variants identified by whole genome sequencing

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Introduction: Oesophageal atresia (OA) is the most common congenital anomaly of the oesophagus with an incidence of around 1/3500 births. OA with a low tracheoesophageal fistula constitute 85% of all cases and syndromic OA at least 50% of cases. Genetic variants have been identified in a proportion of patients with syndromic OA but the genetics behind isolated forms remains elusive.

Materials and Methods: We identified three families with recurrent and isolated OA suggesting inherited factors behind the malformation. Whole genome sequencing (WGS; Illumina) was performed on DNA from affected individuals ($n = 6$) and presumed obligate and healthy carriers ($n = 4$).

Results and Conclusions: We identified single nucleotide variants (SNVs) and small insertions/deletions (indels) using GATK, and structural variants (SVs) using Manta. After stringent filtering (frequency = 0) against the Swedish 1000 genome project (SweGen), only one gene (*DOCK4)* remained that contains rare SNVs in all 10 individuals. Furthermore, we identified a total of 186 SVs in the affected individuals. Interestingly, we observe a significant enrichment of rare SVs on chromosome 21, suggesting a role for this chromosome in the aetiology of isolated OA in our cohort.

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P03.36C

Next Generation Sequencing (NGS) differential genetic analysis in polycystic kidney diseases: work in progress

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Polycystic Kidney Diseases are clinically and genetically heterogeneous conditions mainly caused by mutations in PKD1/2. In the absence of a family history and/or in the presence of atypical presentations, other cystic diseases or other clinical conditions should be considered for a differential diagnosis. Thanks to NGS is now possible to modulate genetic test ranging from a few genes to a comprehensive clinical-exome testing, allowing differential genetic analysis based on the clinical suspicion. To evaluate this approach, we applied the illumina Clinical-Exome NGS protocol in 10 patients negative for ADPKD. Analysis of 106 genes, selected due to their relation with atypical form of PKD, was performed using an in-house validated pipeline (100% sensitivity, >94% specificity). Considering a population frequency <1% in the 1000KG and Exac Databases and basing on the consistency with the clinical phenotypes we focused on 16 candidate variants in total. Eight of these variants were private and novel. We found: 4 novel variants in HNF1B, BBS9, REN, GLIS3 and 5 already described variants in BBS9, PKHD1, GLIS3, NPHP3 and WDPCP. Moreover, one novel stop-gained and one start-lost variant were found in INVS and GLIS3 and three intronic and one splice donor variants were found in TMEM138, TMEM67, IFT43 and COL4A1. Interestingly, one frameshift variant was found in PKD1, previously misdiagnosed by Sanger sequencing, indicating a good performance of NGS. All variants, except one (start-lost variant in GLIS3), were confirmed by Sanger sequencing as well as the re-evaluation of clinical phenotypes and the potential for family and functional studies.

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P03.37D

A comprehensive genetic testing in children with the clinical diagnosis of ARPKD identifies phenocopies

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Introduction: Autosomal recessive polycystic kidney disease (ARPKD) is genetically one of the least heterogeneous ciliopathies, resulting primarily from mutations of *PKHD1*. However, 15-40% of patients diagnosed with ARPKD are found not to carry *PKHD1* mutations. Aim of this study was to identify whether mutations in other genes might explain these cases.

Materials and Methods: Thirty-six unrelated patients with the clinical diagnosis of ARPKD were tested by *PKHD1* sequencing and MLPA. Patients without biallelic mutations were reevaluated and tested for second locus mutations in function of the phenotype, followed, if negative, by clinical exome sequencing.

Results: Twenty-eight patients (78%) carried *PKHD1* point mutations, three of whom were heterozygotes for small-scale alterations. Two of the three patients possessed either a duplication of exons 33-35 or a large deletion involving exons 1-55 in trans. All eight patients without *PKHD1* mutations had mutations in other genes (*PKD1* (n = 2), *HNF1B* (n = 3), *NPHP1*, *TMEM67*, *PKD1/TSC2*). Perinatal respiratory failure, a kidney length >+4SD and early-onset hypertension were specific features of *PKHD1*-associated ARPKD.

Conclusions: We found all ARPKD cases without *PKHD1* point mutations to be phenocopies, and none to be explained by biallelic *PKHD1* copy number variations. Based on this non-consanguineous cohort, screening for copy number variations is indicated only in patients with a heterozygous point mutation. This work was supported by OTKA K109076 and Ministry of National Economy, Hungary, GINOP-2.3.2-15-2016-00039 (to István Balogh, Zoltán Maróti and Tibor Kalmár), MTA-SE Lendulet Research Grant (LP2015-11/2015) of Hungarian Academy

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P03.38A

Prader-Willi syndrome: clinical particularities found in 15 patients

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Prader-Willi syndrome (PWS) is a genetic disorder characterized by severe hypotonia and feeding difficulties in early infancy, followed by excessive eating and morbid obesity in childhood, intellectual disability, small hands and feet and hypogonadism. The genetic defect (microdeletion, uniparental disomy or imprinting defect) involves region 15q11.2-q13. We performed a clinical and genetic study of 15 patients diagnosed with PWS in Iaşi Medical Genetics Center to evaluate the relevance of clinical features for the diagnosis, identify particularities and possible complications. All cases were confirmed using MS-MLPA or FISH testing. We have identified neonatal hypotonia in 11/15 cases, feeding difficulties in infancy in 12/15, excessive weight gain in 9/15 (6/15 being under GH therapy), characteristic facial features in 13/15 and acromicria in 8/15. Micropenis (2/6), cryptorchidism (4/6) and hypoplasia of labia minora (1/9) indicated the presence of hypogenitalism. High pain threshold was seen in 5/15 cases, sleep apnea as well as skin picking in 4/15, ADHD, strabismus and incontinence were associated in 2/15. One case associated central fever. Particular features identified included: skeletal anomalies (5/15), congenital heart defects (4/15), cerebral malformations (3/15), ADHD (2/15), epilepsy (1/15) and multiple allergies (1/15). The correlation of the clinical features with the type of genetic defect will be illustrated in detail. Follow-up revealed: skeletal deformities that contraindicated GH therapy in one case, epilepsy and multiple allergies in another and central fever that led to death in another. In conclusion, we present a clinical study on 15 PWS patients to illustrate particular features and long-time evolution.

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P03.39B

The importance of comprehensive genomic profiling in differential diagnosis and discovery of novel disease causing genetic variants in patients with pediatric lung diseases

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Introduction: Primary ciliary dyskinesia (PCD) is a rare inherited autosomal recessive or X-linked disorder that mainly affects lungs. PCD diagnosis requires well-described clinical phenotype combined with the identification of underlying genetic causes since clinical symptoms of PCD overlaps with symptoms of other pediatric lung diseases. The aim of the study was to point out the significance of comprehensive genomic profiling in differential diagnosis of presumed PCD patients and detection of novel disease-causing genetic variants.

Materials and Methods: Using Next Generation Sequencing (NGS) approach and Clinical-Exome Sequencing Panel with 4813 genes, we analysed 74 genes related to PCD and other pediatric lung diseases in a cohort of 21 Serbian patients with clinically suspected PCD.

Results: After variant filtering and prioritization, the molecular diagnosis of PCD was achieved in 14/21 (66.67%) patients. The most frequently mutated gene was *DNAH5* (23.33%). We have also genetically diagnosed asthma, NRDS, and bronchiectasis without CF, leading to detection rate of 95.24% (20/21). Identified variants were in homozygous, compound heterozygous and trans-heterozygous state. We also detected a novel homozygous frameshift mutation that leads to premature stop codon in *DNAI1* gene (c. 947_948insG, p. Thr318TyrfsTer11), in two affected PCD siblings. For detailed characterization of novel genetic variant we performed RT-qPCR, *in silico* prediction of variant impact at protein level and protein analysis by Western Blot.

Conclusions: Analysis of the genomic profile of PCD suspected patients improves differential diagnosis and enables prenatal and carrier testing. It also leads to better understanding of the molecular basis of pediatric lung diseases.

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P03.40C

PLVAP in protein-losing enteropathy: a homozygous missense variant leads to an attenuated phenotype

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Background: The integrity of the intestinal lymphatics is essential for proper nutrient absorption and tissue homeostasis. Damage to the lymphatic endothelial cells (LECs) leads to an enteric loss of protein-rich lymphatic fluid, i.e. protein-losing enteropathy (PLE). We investigated the genetic cause of PLE in two patients, first-degree cousins once removed, who presented at 22 and 2.5 years.

Methods: Whole exome sequencing was performed for two affected and five healthy family members. Variants were filtered based on autosomal recessive inheritance model, population frequencies, and predicted functional effect.

Results: We identified a rare homozygous variant (NM_031310.2:c.101T>C; p.Leu34Pro) in *PLVAP*, which co-segregated with the disease. The variant is predicted deleterious by bioinformatics algorithms, affecting the protein's transmembrane domain. Electron microscopy (EM) of the younger patient's duodenal biopsy revealed preserved endothelial fenestral diaphragms.

Discussion: The plasmalemma vesicle-associated protein (PLVAP) is the building block of LEC fenestral diaphragms, which serve as a filtration system that blocks passage of large molecules and pathogens through the intestines. Mouse models of *Plvap*-deficiency demonstrate PLVAP's important contribution to LEC barrier function. Recently, a homozygous nonsense variant in *PLVAP* was reported in a single patient with severe congenital PLE and hypertriglyceridemia. We now show that bi-allelic missense variants in *PLVAP* can cause an attenuated form of the syndrome, albeit apparently intact endothelial diaphragms on EM. Thus, our report establishes the role of PLVAP in the pathophysiology of PLE, and expands the phenotypic and mutation spectrums of the disorder, underscoring the importance of PLVAP in LEC barrier function in the gut.

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P03.42A

Genes with mutational enrichment in metastatic clear cell kidney cancer associate with patient mortality

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Introduction: Kidney cancer only represents 2-3% of all cancers. However, more than 60% of the patients die after 2-3 years from diagnosis. Here we aimed to evaluate the association of somatic genetic variation with mortality by metastatic kidney cancer.

Materials and Methods: Whole-exome sequencing from paired tumor/normal FFPE tissue obtained from ten patients with metastatic clear-cell renal cell carcinoma was performed with AmpliSeq Exome RDY kit with the Ion Proton System (Thermo Fisher Scientific). Somatypus and VEP were used to identify and annotate single nucleotide variants and small indels. A gene-based burden of putative somatic mutations was calculated, and mutation enrichment assessed by the Fisher's exact test. Gene-set enrichment analysis (GSEA) was evaluated with EnrichR.

Results: Mean exome-wide target coverage was 127-fold for tumor and 130-fold for matched normal samples. A total of 9,220 putative somatic variants mapping to 5,256 genes

were detected across samples. Mutation enrichment was associated with mortality at nominal significance in 138 genes (lowest p = 2.0e-6), and GSEA on this subset evidenced a significant link with renal cancer (p = 2.3e-9), specifically for 49 of the genes.

Conclusions: Mutational burden in 138 genes associate with mortality among metastatic kidney cancer patients, which represents a concise gene set for conducting focused validation and functional studies.

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P03.43B

Lung metagenomics to predict patient mortality by non-pulmonary sepsis

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Introduction: Death by sepsis remains high in patients admitted into intensive care units (ICUs) worldwide. Identifying early biomarkers of disease prognosis remains an urgent necessity. Here we hypothesized that lung microbiome perturbations associate with ICU mortality in septic patients.

Materials and Methods: We analyzed DNA from 41 bronchoalveolar aspirates from non-pulmonary septic ICU patients at sepsis diagnosis (8h), and after 24h and 72h. Bacterial abundance was obtained by DNA sequencing of the 16S rRNA V4. QIIME was used for operative taxonomic units (OTUs) assignment.

Results: The core microbiome (taxa in >50% of patients) was composed by 46 OTUs and its number diminished in deceased compared to surviving patients at all collection times. Diversity differences were evident very early (17 *vs* 45 OTUs in deceased and survivors at 8h of sepsis diagnosis, respectively; $p < 0.01$). The four most abundant taxa in non-survivors comprised >89% of the core microbiome, while these only represented 15% of the core microbiome in survivors.

Conclusions: A reduction in bacterial lung diversity within 8h of sepsis diagnosis associates with ICU mortality, providing a novel prognostic biomarker at an early stage of disease.

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P03.45D

Searching for causal treatment in patients with Wolfram syndrome

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Introduction: Wolfram syndrome (WFS) is an example of neurodegenerative disease due to increased ER stress coexisting with diabetes mellitus with no causal treatment. WFS is caused by recessive mutations in the *WFS1* gene among which are the mutations of premature termination codons (PTCs). Some prospects for the causal treatment of WFS patients can give a readthrough of PTCs. The use of a chemical compound e.g. PTC124 can result in bypassing the PTCs and a continuation of translation. The aim of the study was to evaluate a repairing of a genetic defect by using PTC124 in WFS patients with confirmed mutations of PTCs.

Materials and Methods: Diagnosis of WFS was confirmed by direct sequencing of the *WFS1* gene. On fibroblasts from skin biopsies of WFS patients and healthy individuals the *in vitro* studies were performed involving the induction of ER stress (Tunicamycin) with a subsequent using a compound PTC124 (Ataluren). Evaluation of ER stress induction was performed by mRNA expression analysis of wolframin and markers of the ER stress (7900HT Real Time PCR; Applied Biosystems, USA).

Results: In 12/15 of WFS patients the mutations of PTCs were identified. The most specific markers of ER stress in patients with WFS should be considered: GRP78, GADD153 (CHOP) and ATF4. Thus, a compound PTC124 may unfortunately increase the ER stress.

Conclusions: It seems that PTC124 by ER stress increasing can not be used as a potential causal treatment for the WFS patients.

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P03.46A
Afifty-year follow-up of familial Hirschsprung disease

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We describe familial Hirschsprung disease (OMIM 142623) with histologically proven congenital intestinal aganglionosis in the proposita, her two affected brothers, her two affected daughters and in the extended family three first cousins (two males and one female). Originally this family was described in 1967 as part of systematic study of the genetics of Hirschsprung disease (Family 21 in E. Passarge, New Eng J Med 1967; 276:138-143). It presumably involves the highest number of affected members documented. In all patients the non-syndromic long segment disease (HSCR type 2) is present. At the age of 8 months the proposita was admitted to Cincinnati Children´s Hospital for ileostomy under the care of one of the authors (EP), then a resident in pediatrics. During the following 15 years she required 41 surgical procedures of the GI tract or the rectum. In spite of the complicated course she is well adjusted to her disorder. This family was re-investigated in November 2017, including two daughters of the proposita also affected with long segment Hirschsprung disease. Both daughters also required several additional surgical procedures. Hirschsprung disease, a genetically heterogeneous and clinically variable disorder, results from the absence or malfunction of intestinal ganglion cells. At least three signal effector pathways are required for normal migration and development of intramural intestinal ganglion cells, (i) the RET tyrosine-kinase receptor and its ligand GDNF (glial-cell-derived neurotrophic factor, OMIM 600837), (ii) endothelin type B receptor (EDNRB, OMIM 600837) and its ligand EDN3 (endothelin 3, OMIM 131244), (iii) SOX10 transcription factor (OMIM 602229).

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P04 Skeletal, connective tissue, ectodermal and skin disorders

P04.01A
Microduplication of 13q31.3 region: clinical and molecular analysis based on a new case

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Microduplication of 13q31.3 is a rare genomic disorder associated with abnormal growth and skeletal development. To date, less than ten comparable submicroscopic duplications involving this region have been described. The most important phenotypic features include developmental delay, digital malformations, growth abnormalities, macrocephaly and facial dysmorphism. *MIR17HG* (encoding the miR-17~92 polycistronic miRNa cluster) and *GPC5* are the major candidate genes for the genotype-phenotype correlation.

Here, we report on the case of a 3 4/12 year-old girl referred for genetic counseling because of excessive height and weight (above the 97th centile) and dysmorphic features (macrocephaly, frontal bossing, hyperthelorism, long palpebral fissures, long philtrum, tilted upper lip, pug

nose, widely spaced teeth). In the neonatal period umbilical hernia was observed. At the clinical examination, indistinct speech, laxity of hand joints, long and overlapping toes, café au lait spot on the thigh and hypoplastic nipples were noted.

Conventional G-banding karyotyping revealed a de novo translocation 46,XX,t(1;13)(q21;q13.31). Whole-genome oligonucleotide microarray analysis revealed a 11.47-Mb cryptic interstitial duplication of the 13q31.1q32.1 region associated with the translocation breakpoints (chr13:85583670-97054435; GRCh37). Microarray studies in both parents gave normal results, proving de novo occurrence of this aberration in the child.

In conclusion, this study contributes additional information for the 13q31.3 microduplication and strongly support the involvement of miR-17~92 cluster of miRNA and *GPC5* gene in normal growth and skeletal development.

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P04.02B

Generation and validation of the first complete knockout model of *abcc6a* in zebrafish

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Introduction: Pseudoxanthoma elasticum is an ectopic mineralization disease due to biallelic *ABCC6* mutations. As no curative therapy is available, development of reliable disease models for compound screening is necessary. Zebrafish, which have a functional *abcc6a* orthologue, are an excellent candidate model organism, if it has a reliable phenotype. For a morpholino-induced knockdown and a missense mutant model of *abcc6a* phenotypic discrepancies are reported, questioning their validity. Therefore, we developed a complete CRISPR/Cas9 knockout model and compared its phenotype to a novel mutant (Sa963) and our morpholino model.

Materials and Methods: Both carriers of the CRISPR-induced (c.180delTCGG) and Sa963 (c.2250+1G>A) heterozygotes were out- and incrossed to F4 generations. Morpholino dosage was optimized to 3ng and co-injected with 4.5ng p53-morpholino. Development was monitored during embryonic development into adulthood. Mineralization was assessed via alizarin red S staining and ImageJ analysis.

Results: In all models we noted similar extensive and progressive vertebral hypermineralization (Table 1), starting during embryonic development, with spinal deformities and scoliosis in adults. Contrary to previously reported data, *abcc6a* was essential for neither embryonic survival nor morphological development.

Conclusions: Observing an identical ossification phenotype in 3 independent models confirms its specificity and indicates for the first time a direct relation between abcc6a deficiency and dysregulated osteogenesis. The phenotype is readily quantifiable and an excellent readout for compound screening.

Table 1: Mineralization semi-quantified at 10 days post fertilization (Mean ± SD; *P<0.05)

	$^{+}/^{+}$ or Control	$^{-}/^{-}$ or Morpholino
CRISPR (c.180delTCGG)	100% ± 9.6	121% ± 8.8 *
Sa963 (c.2250+1G>A)	100% ± 15.8	135% ± 40.2 *
Morpholino *abcc6a*	100% ± 19.2	155% ± 29.6 *

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P04.03C

Functional characterization of a predicted regulatory element associated with alopecia areata (AA)

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Alopecia areata (AA) is a genetic complex hair loss disorder characterized by patchy hair loss. Genetic studies have supported the hypothesis that AA is autoimmune in nature. The identified loci only explain a limited proportion of disease heritability. The goal of the current project is to functionally characterize a regulatory element (RE) and its effect on IL2RA and other genes in close vicinity. Based on data of a recent meta-analysis on AA, we performed a genome-wide in-silico analysis of REs. We cloned a T-cell specific RE, which contains the identified lead SNP, into a pGL4.23 vector to perform dual-luciferase-assay (DLA) in Jurkat cells. Further, we analyzed the effect of the reference and alternative allele on gene transcription in association with a minimal promoter or with a promoter of a predicted target gene.

We identified a region mapping into a predicted T-cell enhancer, which might influence the expression of the nearby AA-susceptibility gene IL2RA. Analysis of CTCF sites revealed co-localization of IL2RA and the RE in the same CTCF flanked domain. DLAs of the cloned RE showed significant decrease of gene expression compared to controls. Furthermore, gene expression was significantly suppressed after integration of the validated IL2RA promoter. However, introduction of the identified SNP led to significantly less suppression.

Taken together, with our experiments we could prove allele-dependent silencing properties of the examined regulatory region. Regulation of the IL2RA gene might play a relevant role in the development of regulatory and effector T-cells in AA.

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P04.04D

A homozygous missense change in canine *ALPL*, an animal model for hypophosphatasia

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Introduction: The purebred dogs are affected with similar skeletal disease as humans. In the present study, we have performed clinical, pathological and genetic examinations to characterize a previously unknown, autosomal recessive skeletal disease in the Karelian Bear Dog (KBD) breed.

Materials and Methods: Clinical examinations were performed on six affected dogs and pathological examination on seven dogs. Exome sequencing was carried out on one affected dog.

Results: Altogether seven affected puppies were recognized in the KBDs. The clinical phenotype varied from a failure to thrive and seizures at 2 weeks of age to a growth defect and severe ambulatory difficulties at 12 weeks of age. Serum analysis indicated low alkaline phosphatase activity and hypercalcemia. Radiographic and pathological examinations revealed defective skeletal mineralization and ossification. Exome sequencing identified a homozygous missense variant in a conserved domain of the tissue-nonspecific alkaline phosphatase gene, *ALPL*. The *ALPL* variant showed full segregation with the disease in a cohort of 489 KBDs, and was absent from 303 dogs from control breeds.

Conclusions: In humans, near 350 *ALPL* mutations are known to cause hypophosphatasia, a metabolic bone disease with heterogeneous clinical manifestations. We have identified a recessive *ALPL* missense change in dogs with clinical and pathological findings compatible with hypophosphatasia. To our knowledge, this is the first time naturally-occurring inherited hypophosphatasia has been described in animals, and we hope our findings contribute to understanding of the disease.

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P04.05A

Loss of GPNMB causes autosomal recessive amyloidosis cutis dyschromica in humans

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Amyloidosis cutis dyschromica (ACD) is a distinct form of primary cutaneous amyloidosis characterized by generalized hyperpigmentation mottled with small hypopigmented macules on the trunks and limbs. Families and sporadic cases have been reported predominantly in East and Southeast Asian ethnicities; however, the genetic cause has not been elucidated. Homozygous premature nonsense mutations leading to loss of function of GPNMB contribute to the iris pigment dispersion phenotype in mouse pigmentary glaucoma. However, no mutations in *GPNMB* have been identified in human pigmentary glaucoma and pigment dispersion syndrome. We establish that the compound heterozygosity or homozygosity of *GPNMB* truncating alleles is the cause of autosomal recessive ACD. Six nonsense or frameshift mutations were identified in nine individuals diagnosed with ACD. Immunofluorescence analysis of skin

biopsies showed that GPNMB is expressed in all epidermal cells, with the highest staining observed in melanocytes. GPNMB staining is significantly reduced in the lesional skin of affected individuals. Hyperpigmented lesions exhibited significantly increased amounts of DNA/keratin-positive amyloid deposits in the papillary dermis and infiltrating macrophages compared with hypo-/depigemented macules. Depigmentation of the lesions was attributable to loss of melanocytes. Intracytoplamic fibrillary aggregates were observed in keratinocytes scattered in the lesional epidermis. Thus, our analysis indicates that loss of GPNMB, which has been implicated in melanosome formation, autophagy, phagocytosis, tissue repair, and negative regulation of inflammation, underlies autosomal recessive ACD, and provides insights into the etiology of amyloidosis and pigment dyschromia.

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P04.06B

MetaXcan gene-based association analysis yields novel insights into male-pattern baldness biology

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Male-pattern baldness (MPB) is a heritable trait and GWAS have implicated more than 100 genomic regions. The majority of associated variants however are located in noncoding regions and their functional relevance remains unclear. Integrative analyses that link genetic variation with biological function are needed to identify relevant genes and mechanisms. Here, we used MetaXcan (Barbeira *et al*., bioRxiv) on summary statistics from two published large-scale genetic studies for MPB (Heilmann-Heimbach *et al*., 2017; Hagenaars *et al*., 2017) and gene-expression data from human hair follicle (own data) and the GTEx-project (skin, adipose tissue, blood) to test for an association between MPB and the genetically determined regulation of gene-expression in these tissues. The analysis identified associations between MPB and expression levels of 30 (hair follicle) to 637 genes (skin). Twenty-seven genes showed significant association in one or more tissues after Bonferroni correction ($P<0.05/27300$ gene-tissue pairs). These included previously implicated genes (e.g. *WNT10A*, *IRF4, TWIST1*) and novel candidate genes such as *SPRR1B* and *TRADD*, which have a reported role in keratinocyte biology and apoptosis, thereby rendering an involvement of these processes in early MPB-pathogenesis likely. The fact that associations with previously implicated genes were detected in subcutaneous adipose tissue (*TWIST1*) and blood (*WNT10A*) lends further support to the hypothesis, that cells in the perifollicular environment play a role in MPB. In summary, our analyses provide evidence for an MPB-associated genetic regulation of plausible candidate genes (*SPRR1B*,*TRADD*,*WNT10A*) and biological processes (apoptosis,adipogenesis) and yield novel insights into the functional effects of current GWAS findings.

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P04.07C

Evidence for a functional interaction between *WNT10A* and EBF1 in the development of androgenetic alopecia (AGA)

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The *WNT10A*-locus on 2q35 is a known risk locus for AGA. A reduced *WNT10A* expression in hair follicle (HF) of risk allele carriers for rs7349332 supports the role of *WNT10A* as the functionally relevant gene (Heilmann et al., 2013). This lead variant is in high LD ($r^2=0.96$, $D'=0.99$) with rs3856551, located within a binding site for the transcription factor EBF1. Interestingly, the gene encoding EBF1 is located at the 5q33.3-AGA risk locus, suggesting that changes in EBF1 mediated regulation of *WNT10A* expression may contribute to AGA. To investigate this potential interaction, we performed luciferase assays by co-transfecting (i) luciferase vectors containing the *WNT10A* promoter and the 2q35-EBF1 binding site with either the risk or the alternate allele and (ii) an EBF1 expression vector. Our experiments in HEK cells showed that EBF1 activates the *WNT10A* promoter and that the *WNT10A*/EBF1 interaction was increased with the risk allele, which together with the previous mRNA expression data suggests

that EBF1 acts as a negative regulator of *WNT10A*. To confirm this interaction in AGA relevant tissue, we tested for co-expression of *WNT10A* and *EBF1* in a published RNA-Seq dataset of mouse HF (Joost et al., 2016) and performed immunofluorescence co-staining in human HF and skin. The analyses revealed the strongest co-expression in HF keratinocytes. Therefore, the initial luciferase assays are repeated in human keratinocytes. In parallel, *EBF1* knockout experiments in human HF are under way. Taken together, our data provide the first evidence for a functional interaction between *WNT10A* and EBF1 in AGA pathobiology.

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P04.08D

Genetic investigation of a rare form of severe tooth agenesis: Anodontia

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Introduction: Andontia refers to the total absence of dentition. It can affect permanent and/or temporary dentition. In most cases, anodontia is associated with different clinical manifestations as a part of syndrome but also non-syndromic forms have been described although their etiology is often unclear.

Material and Methods: In this study we explored a Tunisian family with most likely a recessive form of anodontia of permanent dentition in the proband whereas the parents and some other family members show hypodontia. We performed DNA targeted sequencing of candidate genes, *PAX9, MSX1, WNT10A* and *AXIN2* and consequently whole exome sequencing (WES).

Results: Sequencing of the 4 genes did not reveal any causative mutation. Therefore, we performed WES on the anodontia patient. We selected six homozygous mutations and segregation analysis on the remaining family members. One of these is a missense mutation located in *NFRKB* gene (Nuclear Factor Related to Kappa-B-binding protein) in the proband. Interestingly, in the rest of the family this mutation is compound heterogeneous with most likely a duplication of the *NFRKB* gene on the other allele. This mutation, predicted to be disease causing, is located at the NFRKB-WHL domain which belongs to a group of helical domains involved in protein-protein interactions. This might suggest that the interaction activity is probably altered in the first stages of development.

Conclusions: This study revealed for the first time the involvement of the *NFRKB* gene in tooth agenesis. NFRKB might be involved in transcriptional regulation. Further qPCR studies and functional studies will be performed.

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P04.09A

Novel heterozygous deletion in *PIEZO2* identified by NGS in a familial case with distal arthrogryposis type 5

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Introduction: Distal arthrogryposis type 5 (DA5) is characterized by multiple congenital contractures of distal extremities associated with ocular abnormalities, facial dysmorphism and restrictive lung disease and caused by heterozygous *PIEZO2* gain of function mutations.

Case report: A 4 year old boy presented with severe club foot, camptodactyly, short stature, ophtalmoplegia, deep-set eyes and micrognathia. The family history revealed that his father had congenital arthrogryposis with short stature, keratoconus and astigmatism. The clinical presentation being very suggestive for DA5. Sanger sequencing of *PIEZO2* was performed initially and revealed no mutation. NGS panel for distal arthrogryposis did also reveal no mutation in the analyzed genes. As no MLPA is available for *PIEZO2*, *in silico* CNV analysis was performed and detected a deletion of exons 32 and 33 in *PIEZO2* (c.4634-?_4855+?del). The deletion was confirmed by RT-qPCR.

Discussion: Heterozygous gain of function mutations in *PIEZO2* cause DA5, Gordon and Marden-Walker syndrome whereas homozygous loss of function mutations cause muscular atrophy, perinatal respiratory distress, progressive arthrogryposis, scoliosis and loss of proprioception. We hypothesize that the in frame deletion of exons 32 and 33 causes a dominant negative effect resulting in a gain of function of the channel activity.

Conclusion: This is the first report of an intragenic deletion in *PIEZO2* in a family with autosomal dominant DA5 diagnosed by NGS. Inframe deletions of *PIEZO2* might be a frequent cause of DA5. As no MLPA is available

for *PIEZO2*, *in silico* CNV analysis using NGS may be a useful tool to diagnose intragenic deletions.

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P04.10B

Camptodactyly-arthropathy-coxa vara-pericarditis syndrome in a large family: A clinical condition with a diagnostic challange

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Introduction: Camptodactyly-arthropathy-coxa vara-pericarditis syndrome (CACP; OMIM 208250) is a rare autosomal recessive disorder caused by *PRG4* mutations. *PRG4* product is a glycoprotein called lubricin. We report on the clinical and molecular findings of two related families who were initially diagnosed with JIA, and *PRG4* mutations were identified in the follow-up.

Materials and Methods: After the referral of the first family with three affected individuals with CACP, a linkage analysis was done and a high LOD score on the long arm of chromosome 1 was found. However, no further studies could be performed, and the family was lost in the follow-up. Years later, and after the discovery of *PRG4* mutations in CACP, another patient from another family with a diagnosis of JIA who was not responsive to anti-rheumatic treatment was referred with a suspicion of a genetic disorder of the skeleton. With the help of the pedigree analysis, it was revealed that these two families were actually related, and a recent contact with the first family could be established years after.

Results: Molecular analysis in all patients revealed a homozygous deletion of two nucleotide (c.1910_1911delCT) in the highly repetitive region of exon 6 of *PRG4* in all patients.

Conclusions: Patients with CACP syndrome can easily be misdiagnosed as JIA, leading to unnecessary treatment and a delay for the actual diagnosis. In patients with arthropathic conditions, CACP should be kept in mind, especially in the presence of progressive symptoms and parental consanguinity.

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P04.11C

The effects of calcium-sensing receptor CASR genotypes, treatment duration, gender bone health and mineral metabolism in chronic renal failure patients

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Introduction: The aim of this study is to examine the effect of the CaSR genotype, which significantly affects Ca, P, PTH values on the disease progression in chronic renal failure (CRF) patients.

Materials and Methods: Fortyfıve CRF patients on hemodialysis treatment aged between 15-80yrs who admitted between 1994-2017 were included. Serum PTH, Ca^{2+} and P values were examined. The control group included 50 healthy patients without CRF, bone disease and osteoporotic risk factors. Genomic DNA isolation was performed using the Gentra Qiagen kit. CaSR allele-specific PCR analysis was performed.Findings: Patients' average age was 60.5 ± 17.5 years, (male/ female 53.3% / 46.7%) Patients with hemodialysis treatment for less than 3 years had normal CaxP value while this value was increased for patients with hemodialysis over 3 years. The pathological CaxP value was found in 8,3% (n = 2/24) of the male patients and 38,09% (n = 8/21) of the female patients (p = 0,029). In the patient group, the 990th codon AA polymorphism in the CaSR gene was found to be 32.4% (n = 12) while in the control group it was 23.1% (n = 6).

Conclusion: The CaSR kodon 990 AA allele seemed to be a risk factor for bone health in our patient group. Among CRF patients with increased hemodialysis duration osteoporosis and fracture risks were increased. Risk of developing bone disease for female patients was higher than that of males.

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P04.12D

WES in 42 trios of syndromic and isolated Chiari Malformation type 1: how to define the genetic cause in a high clinical heterogeneous condition

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Chiari malformation type 1 (CM1) is a congenital anomaly of cranio-cerebral junctions characterized by underdevelopment of the occipital bone and posterior fossa (PF) and consequent cerebellar tonsil herniation across the foramen magnum. This condition can impair the normal flow of cerebral spinal fluid (CSF) leading to syringomyelia. Patients display a high degree of clinical variability depending on the compression of the tissue, nerves and on the buildup of CSF pressure. CM1 is also associated with known syndromes, (e.g. craniosynostosis), and is often reported as clinical sign in more complex phenotypes, but the molecular mechanisms of isolated CM1 are not yet known. To understand the molecular basis and which factors could contribute to the high heterogeneity, we performed WES of 42 trios with sporadic and syndromic, without craniosynostosis, CM1. WES of syndromic cases provided a diagnosis of distinct genetic disorders, in which pathogenic variants were associated to bone dysplasia, growth retardation and resistance to gonadotropins. In sporadic cases, variants in genes involved in common molecular pathways have been identified; these are all associated with modeling and deposition of bone matrix and with regulatory processes, all requested in the same pathway. In 75% of trios we found variants in the same recurrent genes, shared both by isolated and syndromic CM1 cases; functional tests on bone biopsies of these patients are underway to demonstrate the pathogenicity of these genes. Our data remark complex interactions among several genes involved in different steps of the same pathways, underlying the high clinical-genetic heterogeneity of CM1.

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P04.13A

Exome sequencing of two Italian pedigrees with non isolated Chiari malformation type I reveals candidate genes for cranio-facial development

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Chiari malformation type I (CMI) is a congenital abnormality of the cranio-cerebral junction with an incidence of 1 in 1280. CMI is characterized by underdevelopment of the occipital bone and posterior fossa (PF) and consequent cerebellar tonsil herniation. The presence for a genetic basis to CMI is supported by many lines of evidence. The cellular and molecular mechanisms leading to CM1 are poorly understood. The occipital bone formation is dependent on complex interactions between genes and molecules with pathologies resulting from disruption of this delicate process. Whole-exome sequencing of affected and not affected individuals from two Italian families with non isolated CMI was undertaken. Single nucleotide and short insertion-deletion variants were prioritized using KGGSeq-knowledge-based platform. We identified three heterozygous missense variants: *DKK1* c121G>A (p.(A41T)) in the first family, and the *LRP4* c.2552C>G (p.(T851R)) and *BMP1* c.941G>A (p.(R314H)) in the second family. The variants were located at highly conserved residues, segregated with the disease, but they were not observed in more than 100 unaffected in-house controls. *DKK1* encodes for a potent soluble WNT inhibitor that binds to LRP5 and LRP6, and is itself regulated by BMPs. DKK1 is required for embryonic head development and patterning. LRP4 is a novel osteoblast expressed receptor for DKK1 and a WNT and BMP signaling pathways integrator. Screening of *DKK1* in a cohort of 65 CMI sporadic patients identified another missense variant, the c.359G>T (p.(R120L)), in two unrelated patients. These findings implicated the WNT signaling in the correct development of the cranial mesenchyme originating the PF.

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P04.14B

A small homozygous *CHST11* deletion in chondrodysplasia, brachydactyly, overriding digits, clino-symphalangism and synpolydactyly

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Introduction: CHST11 is a membrane protein of Golgi that catalyses the transfer of sulphate to position 4 of the N-acetylgalactosamine residue of chondroitin. Chondroitin sulphate is the predominant proteoglycan in cartilage, and its sulphation is important in the developing growth plate of cartilage. A homozygous deletion encompassing part of the gene and the embedded microRNA *MIR3922* had been detected in a woman with hand/foot malformation and malignant lymphoproliferative disease. Chst11 deficient mouse has severe chondrodysplasia, congenital arthritis and neonatal lethality. We searched for the causative variant for the unusual combination of limb malformations with variable expressivity accompanied by skeletal defects in a consanguineous Pakistani kindred.

Materials and Methods: We performed detailed clinical investigations in family members. Homozygosity mapping using SNP genotype data was performed to map the disease locus and exome sequencing to identify the underlying molecular defect.

Results: The limb malformations include brachydactyly, overriding digits and clino-symphalangism in hands and feet, and syndactyly and hexadactyly in feet. Skeletal defects include scoliosis, dislocated patellae and fibulae, and pectus excavatum. The disease locus is mapped to a 1.6-Mb region at 12q23, harbouring a homozygous in-frame deletion of 15 nucleotides in *CHST11*. Novel variant c.467_481del (p.L156_N160del) is deduced to lead to the deletion of five evolutionarily highly conserved amino acids and predicted as damaging to protein by *in silico* analysis.

Conclusions: Our findings confirm the crucial role of CHST11 in skeletal morphogenesis and show that *CHST11* defects have variable manifestations that include a variety of limb malformations and skeletal defects.

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P04.15C

Regenerative approach for treating maxillofacial defects associated with some genetic disorders

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Introduction: Cleft palate is one of the most common congenital craniofacial defects that may present alone or in association with various genetic disorders. Repairing such defect is very important to restore oral functions and normal facial features. Regenerative medicine and stem cell therapy are emerging fields that have shown great potentials in treating various diseases. Here, we follow a regenerative approach by introducing a method in which we use autologous bone marrow mononuclear cells (BMMNCs) combined with platelet-rich fibrin (PRF) and nano-hydroxyapatite for bone regeneration in patients with unilateral alveolar clefts.

Materials and Methods: The study included 10 patients with unilateral alveolar cleft defects and of age range 8 to 15 years. Autologous BMMNCs were isolated using the density gradient separation method and seeded on a collagen sponge. The seeded collagen sponge was then used in combination with nano-hydroxyapatite and autologous PRF to repair alveolar cleft defects via the regenerative approach. The effectiveness of the technique was evaluated after 12 months of follow up via clinical and radiographic assessments.

Results: All patients healed probably without any complications. During the 12-month follow-up, no donor site morbidities have been reported. Postoperative pain, bleeding, and swelling were within the normal for same surgical procedures. Cone beam radiographs showed successful complete bone regeneration in all cases.

Conclusion: Results of this study suggest that a mixture of autologous BMMNCs, nano-hydroxyapatite, and PRF greatly promote bone regeneration providing a novel therapeutic strategy for alveolar cleft defects.

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P04.16D

Expanded phenotypic spectrum of type I collagenopathy

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It is known that type I collagenopathy has a broad-spectrum phenotypic variability. Here, we report a case of a Korean girl with a heterozygous *COL1A1* mutation who had an atypical presentation. A 26-month-old girl presented with

delayed motor development and failure to thrive. She had severe growth retardation. She exhibited right-sided plagiocephaly, blue sclerae, and facial dysmorphism, including a small pointed chin, frontal bossing, and a triangular face, but had microcephaly. Whole-exome sequencing revealed a novel *de novo* heterozygous sequence variant in *COL1A1* (p.Gly1127Asp), which was validated by Sanger sequencing. Radiological finding showed generalized osteoporosis with progressive scoliosis of the spine without evidence of platyspondyly related to fractures and bowing of the long bones, and markedly delayed carpal bone age. Muscle pathology showed a marked size variation of myofibers and selective type 1 atrophy. This study expanded the clinical and genetic spectrum of type I collagenopathy with a *COL1A1* variant. Therefore, we suggest that type I collagenopathy should be considered in the patients who have some features of osteogenesis imperfecta simultaneously with atypical features such as facial dysmorphism.

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P04.17A

Whole exome sequencing identifies mutations in 10% of familial non-syndromic cleft lip and/or palate patients in genes mutated in well-known syndromes

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Introduction: Oral clefts i.e. clefts of the lip and/or cleft palate (CL/P) are the most common craniofacial birth defects with an approximate incidence of ~1/700. To date physicians stratify patients with oral clefts into either syndromic CL/P (syCL/P) or non-syndromic CL/P (nsCL/P) depending on whether the CL/P is associated with another anomaly or not. In general, patients with syCL/P follow Mendelian inheritance, while those with nsCL/P, have a complex etiology and as such, do not adhere to Mendelian inheritance. Genome-wide association studies (GWAS) have identified approximately 30 risk loci for nsCL/P, which could explain a small fraction of heritability.

Materials and Methods: To identify variants causing nsCL/P, we conducted Whole Exome Sequencing (WES) on 84 individuals with nsCL/P, drawn from multiplex families (n = 46).

Results: We identified rare damaging variants in four genes known to be mutated in syCL/P: *TP63* (1 family), *TBX1* (1 family), *LRP6* (1 family) and *GRHL3* (2 families), and clinical reassessment confirmed the isolated nature of their CL/P.

Conclusion: These data demonstrate that CL/P patients without cardinal signs of a syndrome may still carry a mutation in a gene linked to syCL/P. Rare coding and non-coding variants in syCL/P genes could in part explain the controversial question of "missing heritability" for nsCL/P. Therefore, gene panels designed for diagnostic testing of syCL/P should be used for nsCL/P patients, especially when there is at least 3rd degree family history. This would allow a more precise management, follow-up and genetic counseling. Moreover, stratified cohorts would allow hunting for genetic modifiers.

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P04.18B

BBS9 as potential tissue-specific key protein in BBSome binding and ciliary trafficking in NCS patients

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Nonsyndromic craniosynostosis (NSC) is a congenital malformation due to the premature ossification of calvarial sutures, with an unclear molecular etiopathogenesis. The Bardet-Biedl Syndrome-associated gene 9 (*BBS9*), already associated to NCS by GWAS, encodes a member of the well-characterized class of BBS proteins that interact through their C-term to form an octameric complex named BBSome, necessary for ciliogenesis and ciliary function. Preliminary data identified a suture specific signature,

including BBS9 and several genes involved in primary cilium signaling and assembly. In particular, we showed the overexpression of 5 spliced isoforms of BBS9 in fused suture specimens of single-suture midline NCS patients. The aim of this study is to clarify the mechanism through which BBS9 exerts its regulatory function during the osteogenic commitment and differentiation of somatic stem cells inside skull bone. We confirmed through qPCR the overexpression of the selected *BBS9* splice isoforms in mesenchymal stromal cells (CMSC) isolated from fused (p) and unfused (n) sutures of NCS patients. Then, we assessed by immunofluorescence that p-CMSC showed a reduced number of primary cilia compared with same patient-n-CMSC. Computational modeling of the upregulated isoforms showed structural differences in the C-term of the proteins, predicting that their binding affinity within the BBSome may be affected. Taken together, these data suggest that selected BBS9 protein isoforms show a tissue-specific increased expression in osteogenic precursors residing in NCS fused sutures, owing to a less effective assembly of the BBSome, which impairs ciliogenesis. [Funding support: NIH-NIDCR grant R01DE16886, Federazione GENE and Università Cattolica]

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P04.19C

IL11RA-related Crouzon-like autosomal recessive craniosynostosis in ten new patients: which are the differences?

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By describing 10 new patients recruited from a population attending centres for Human Genetics, we further delineate the clinical spectrum of a new syndromic craniosynostosis resembling Crouzon syndrome. Singularly, it is inherited according to an autosomal recessive mode of inheritance. Missense mutations in *IL11RA*, a gene encoding alpha subunit of interleukin 11 receptor, were at cause in all five families, four of them not reported, including two in the Ig-like C2-type domain. A subset of patients had an associated connective tissue disorder with joint hypermobility and intervertebral discs fragility. Two of our patients had syringomyelia. A lower figure of teeth anomalies than previously reported in the two large series of patients evaluated in dental institutes points towards an ascertainment bias.

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P04.20D

Targeted exome sequencing analysis in Turkish non-syndromic craniosynostosis patients

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Introduction: Craniosynostosis is described as an early fusion of one or more calvarial sutures. Early closure of these sutures results with new head shape that inhibits the normal growth and development of the brain. Worldwide, the estimated prevalence of the syndrome is 1 in 2500. Craniosynostosis is divided into two sub-goups: syndromic and non-syndromic (nsCRN). More than 70% of the patients have nsCRN. Although more than 50 genes were identified, the genetic cause is mostly unknown. Approximately 24% of cases can be genetically identified.

Method: Unrelated seventeen nsCRN Turkish cases have been sequenced by using Dysmorphia and Dsyplasia Research Panel v2, includes 519 genes, of Ion AmpliSeq. IonS5 sequencing technology was used and data were analyzed with Ingenuity software. Genetic variants in several genes known to cause craniosynostosis were filtered. Clinically pathogenic variants were checked and confirmed by Sanger sequencing.

Results: In six of the cases (35.3%), we identified six different mutations (including three novel ones) in *ERF*, *FREM1* and *TCF12* genes. The novel missense mutations are p.P1802L(c.5405C>T) and p.G1493R(c.4477G>A) in *FREM1*, and frameshift mutation is c.1106_111delCTCTCAC(p.P369fs*26) in *TCF12* gene. POLYPHEN,

PROVEAN and SIFT analysis revealed that p.P1802L and p.G1493R are predicted to be deleterious and damaging with scores of 0.997; -9.07; 0.003 and 1.000; -3.99; 0.019, respectively.

Conclusion: Our data highlights the importance of increased diagnostic rate (35.3%) with the use of this panel, and help to solve the genotype-phenotype correlations in nsCRN.

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P04.21A

Characterization of the calvarial suture skeletogenic stem cell niche in nonsyndromic craniosynostosis

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Nonsyndromic craniosynostosis (NCS) is the congenital premature fusion of skull sutures. The suture mesenchyme houses a skull-specific stem cell niche, plausibly impaired in NCS fused suture sites. To test this hypothesis, we characterized the stem cell niche of open and fused sutures of NCS patients. Lineage-specific markers were analyzed, by qPCR and immunofluorescence, in suture tissues and in calvarial mesenchymal stem cells (CMSC). MSC from alternative tissues served as controls. We analyzed the localization of THY1 (skeletal stemness-marker), GLI1 (putative calvarial stemness-marker) and AXIN2 (mesenchymal cell fate determinant) in suture tissue sections: AXIN2 resulted mainly expressed at the endosteal ossified side, while THY1 and GLI1 were primarily expressed within the trabeculae, enriched with proliferating cells. Both NCS suture tissues and CMSC isolated thereof expressed reduced levels of *TEK* and *ENPEP* (bone marrow stem cells differentiation markers) compared with controls. *AXIN2* levels were higher in open suture-derived CMSC than in fused suture cells and in controls. Upon *in vitro* osteogenic induction, the expression of *THY1* and *GLI1* decreased, whereas *AXIN2* levels increased, in both open- and fused- suture derived CMSC. CMSCs isolated from both fused and unfused sutures shared the same marker expression profile, indicating that explant cultures allowed selecting comparable cell populations, $THY1^{+}/GLI1^{+}$ representing the stem cell population within the human calvarial niche. Our data seem to suggest that in NCS the *in vivo* tissue microenvironment may cause the enhanced osteogenic differentiation of suture MSCs leading to premature suture closure. Funding support: Federazione GENE and Università Cattolica del Sacro Cuore

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P04.22B

The outcome of broad genetic screening in a cohort of 144 patients with craniosynostosis

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Approximately 1 in 2000 children are affected by craniosynostosis. During the last few years, mutations in several genes have been identified as cause of early closing of the cranial sutures. The list increases constantly as new genes with relation to craniosynostosis are detected. Craniosynostosis occurs isolated or associated with other symptoms including malformations as part of several syndromic disorders - the most known are represented by Pfeiffer, Crouzon, Jackson-Weiss, Antley-Bixler, Apert, Saethre-Chotzen and Muenke syndromes. The aim of our project was to study the prevalence and spectrum of the genetic disorders which are associated with early suture closing in a unique patient cohort including 144 individuals with mostly uni- or bicoronal synostosis. The mutational screening of our patient cohort has been performed by using a custom-designed NGS-enrichment panel including 63 craniosynostosis-related genes selected from OMIM. In 91 out of 144 screened patients (approximately 63%), either a known previously reported pathogenic variant or a likely pathogenic variant has been detected. As expected, the majority of variants have occurred in the craniosynostosis "core genes": *FGFR2, TWIST1, FGFR3, TCF12, EFNB1* and *POR*. However, novel likely pathogenic variants have been observed also in *IL11RA, KMT2D* and *SKI*-genes. Our study shows that a broad genetic screening using a targeted NGS assay have a high diagnostic yield in a large cohort of patients with craniosynostosis.

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Ultrastructural elastic fiber morphology in cutis laxa reflects the underlying pathogenesis and supports a novel clinical classification

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Background: Cutis laxa (CL) syndromes are a heterogeneous group of multisystem disorders that share a loose, redundant skin as a common feature reflecting elastic fiber (EF) deficiency. The pathogenesis of each of the CL subtypes is different but affects elastogenesis. However, light microscopy of the dermis is non-discriminative and the recently observed vast molecular heterogeneity mitigates the clinical validity and practicality of the current classification, based on the mode of inheritance and systemic involvement.

Aims: We aim to classify the CL subtypes by means of a simple flowchart and to evaluate correlations between EF ultrastructural morphology (as evaluated by transmission electron microscopy) and clinical presentation.

Results: Following literature review, we developed a 7-step flowchart to classify the CL subtypes. As a proof of principle, we systematically evaluated 68 CL patients from our in-house database and could allocate 95% of patients to the right gene. We performed transmission electron microscopy in skin biopsies of all CL subtypes and found discriminative and specific findings that correlate with the main presenting symptoms (emphysema, arterial tortuosity, skeletal defects/mental disability with or without intrauterine growth retardation/cataract). Moreover, EF ultrastuctural morphology reflects the involved molecular pathogenesis and provides new insights in elastic fiber biogenesis.

Conclusion: Our novel nosology of the CL syndromes provides a practical approach to the broad differential diagnosis of CL syndromes. The classification forms a basis to integrate the clinical presentation with the pathogenesis and ultrastructural EF defects and might bode for new management guidelines and therapeutic approaches.

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Diverse mechanisms of germline and somatic mosaicism in CYLD cutaneous syndrome

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Six unsolved cases that met clinical diagnostic criteria for CYLD cutaneous syndrome, deemed to be mutation negative following Sanger sequencing, were investigated for clinical and genetic features of mosaicism. One case demonstrated 8% blood mosaicism for a pathogenic mutation using a NGS assay targeting *CYLD*. We found no causative lesions in the blood of five of the six remaining cases. In two cases, we investigated tumour tissue. In the first case, two tumour samples demonstrated a recurrent 19bp deletion in the skin only. In the second, five distinct mutations resulting in a stop codon in *CYLD* were detected in five tumours. SNP array analysis of three tumours demonstrated a recurrent 5.5Mb deletion encompassing *CYLD* and 23 other genes. This patient had a daughter who had developmental delay and unilateral renal agenesis, but no cylindromas. This phenotype has been reported in other patients with large germline deletions including *CYLD*, suggesting transmission of the deletion. The remaining

three cases had clinical features suggesting cutaneous mosaicism. We conclude that, firstly, mosaicism may be detectable in the blood and account for the presence of cylindromas in the skin. Secondly, some patients may only have a unilateral linear arrangement or cluster of tumours, with absence of mosaic mutation in the blood suggesting cutaneous mosaicism alone. Thirdly, mosaic mutations detectable in the blood may affect the germline and be transmissible to offspring; where this is due to a contiguous deletion involving *CYLD*, the phenotype may involve multiple organ systems.

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P04.26B

Microbial signatures in TIF1γ autoantibody positive dermatomyositis patients

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Introduction: Tripartite motif-containing (TRIM) proteins are involved in innate immunity. Reports support a role for microbial infections in dermatomyositis (DM); TIF1γ (TRIM33) autoantibody positive patients may have reduced ability to restrict pathogen infection.

Material and Methods: Serum total Ig from 20 DM patients and 20 age-matched healthy controls were pooled for competitive panning and clonal expansion of the FliTrxTM bacterial random-peptide surface display system. DNA libraries were sequenced using pair-end high-throughput sequencing. Translated peptide sequences were searched for maximum exact matches against the NCBI microbial database and assigned to taxa.

Results: DM patients exhibited higher number of microbial peptide reads ($22x10^6$ vs $14x10^6$) and unique microbial taxa ($32x10^5$ vs $28x10^5$). Viral peptides were higher in DM patients ($4x10^6$ vs $2x10^6$) and occupied larger space in the complete microbial IgOme (12% vs 9%). Peptides of cellular microbial origin also were increased in DM patients but with no change in the relative abundance (63% vs 64%). Specific differences were observed for dsDNA (*Herpesvirales*), ssRNA (*Orthomyxoviridae*), and retro-transcribing viruses (HIV) and for potentially pathogenic bacteria (*Streptococcaceae*).

Conclusions: This is the first systematic high-throughput investigation of a link between microbial exposures, TIF1γ autoantibodies and dermatomyositis. The increased presence of viral peptides in DM patients, particularly ssRNA and retro-transcribing viruses, agrees with pathways known to be regulated by TRIMs and points towards increased susceptibility for certain viral families. The increased detection of peptides against potentially 'pathogenic' but not 'non-pathogenic' bacteria from the same families might play a key role in the development of the disease.

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A whole exome study identifies novel candidate genes for vertebral bone marrow signal changes (Modic changes)

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Lumbar disc degeneration (LDD) is one of the contributing factors behind low back pain (LBP). Modic change (MC) is a phenotype of LDD and is visualized as bone marrow signal intensity changes on magnetic resonance imaging. MC is a heritable trait with heritability estimation around 30%. It is strongly associated with LBP. We studied two families to identify predisposing variants for MC. Nine individuals were chosen for whole exome sequencing. We focused on rare (MAF < 0.01) and private variants with harmful *in silico* predictions and variants located in regulatory regions. The identified variants were genotyped from additional family members. One rare variant co-segregated with MC in each family. In the Family I, the observed variant was an insertion and deletion mutation in the *HSPG2* gene, resulting in a premature stop codon. *HSPG2* encodes a heparin sulfate proteoglycan, which is a structural protein expressed in mammalian cartilage and basement membranes. Rare autosomal recessive disorders with osteochondrodysplasia are caused by mutations in the *HSPG2* gene. In the Family II, a single nucleotide polymorphism in the *MAML1* gene was identified. MAML1 is

considered to be a transcriptional coactivator in the Notch signaling pathway which regulates many biological functions including cartilage development and homeostasis. MAML1 has been reported to affect the activity of RUNX2, a transcription factor essential in the osteoblast differentiation. RUNX2 has been reported to be highly expressed in degenerated discs. We identified two promising candidate genes for MC, *HSPG2* and *MAML1*. Our findings are novel in lumbar spine degenerative phenotypes.

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Intrafamilial phenotypic heterogeneity in dominant dystrophic epidermolysis bullosa associated with G2043R mutation in *COL7A1*

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Introduction: Dystrophic epidermolysis bullosa (DEB) is a rare form of genodermatoses characterized by blistering condition, caused by *COL7A1* gene mutations with dominant or recessive inheritance. Autosomal dominant DEB may remain throughout life in a mild form. However, phenotypic aggravation may be seen between patients. Here, we present a family with seven affected members from three-generations with phenotypic heterogeneity.

Materials and Methods: Genomic DNA was isolated from peripheral blood samples of the patients and *COL7A1* gene was sequenced.

Results: *p.G2043R* (*c.6127G>A*) mutation was found in the index patient and verified in the other affected family members. One patient in the family was diagnosed with DEB while others had severe pruritus and lichenified linear plaques located more prominently on the extensor surface of the arms and shins. Disease onset was early infancy with a significant increase in complaints after puberty. In female patients, who had a more pronounced phenotype, pregnancy was associated with exacerbation of the disease. Nail dystrophy of hands and feet was present in all patients. Index patient had moderately high serum IgE levels. Skin biopsy revealed eosinophilic infiltrate, and the previous diagnosis was in favor of acquired epidermolysis bullosa. Intravenous immunoglobulin (IVIG) treatment was initiated to the Index patient; which decreased her generalized lesions and severe pruritus very effectively.

Conclusions: Genetic and environmental factors as well as hormonal status may be responsible for the intrafamilial phenotypic heterogeneity. Also, we would like to emphasize IVIG as a treatment option in DEB patients with more severe phenotype.

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P04.29A

Comparison of the distribution of duplicated regions associated with *SHOX* gene between LWD/ISS patients and population sample - conclusions of the meta-analysis

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Introduction: The human *SHOX* gene encodes an important growth regulating transcription factor. Heterozygous deletions of the gene or deletions of one of its numerous enhancers are responsible for Lėri-Weill dyschondrosteosis (LWD) and small portion of idiopathic short stature (ISS). Effect of reciprocal duplications is less distinct. The aim of our study was to compare frequency, extent and distribution of *SHOX* gene and associated elements duplications between the LWD/ISS patients and population sample. A preliminary analysis indicated that rather than the difference in frequency it is the difference in distribution of duplicated areas. A meta-analysis of published cases was performed to confirm the consistency of these data.

Material and Methods: For the purpose of meta-analysis, merged groups of published cases were created: LWD patients (31 individuals), ISS patients (29 individuals) and population sample (36 individuals). Only carriers of the duplication with one brakepoint within the chromosomal region chrX:398,000-980,000 (hg19) were included. Extent, distribution and relative frequency of duplications were compared among merged groups.

Results: There was a significant difference in the relative frequency of CNE-9 enhancer duplications (11 vs. 3) and complete *SHOX* (exon1-6b) duplications (4 vs. 24) (p-value 0.0139 and p-value 0.000014, respectively) between the merged LWD sample and the merged population sample.

Conclusion: We propose, partial duplications of the *SHOX* gene coding sequences and small duplications encompassing the CNE-9 enhancer are the highly

penetrating alleles associated with the increased risk of LWD/ISS development. *Acknowledgement:* The study was supported by Charles University (UNCE204022) and its Grant Agency (GAUK202615).

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Degradation routes of trafficking-defective VLDLR mutants associated with dysequilibrium syndrome

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Introduction: Endoplasmic reticulum associated degradation (ERAD) of misfolded proteins by the ubiquitin-proteasome system is a recurrent theme in rare genetic disorders and the process crucially involve ER-membrane complexes such as HRD1-SEL1L. Previously, we have reported that missense mutations in the Very Low Density Lipoprotein Receptor gene (*VLDLR*), causing Dysequilibrium syndrome (DES), disrupt ligand-binding, due to retention of the mutants in ER. This study explores in detail the degradation routes of these ER-retained VLDLR mutants.

Materials and Methods: The missense pathogenic *VLDLR* variants have been generated by QuikChange site-directed mutagenesis. The constructs were expressed in HEK293 cells and analyzed by immuno-pull down assays and Western blotting. Protein turn-over studies were conducted by translation shut-off assays and inhibition of proteasomal/lysosomal degradation. The HRD1-SEL1L knockout HEK293 cell lines have been generated by CRISPR/Cas9.

Results: We show that VLDLR mutants are retained in the ER for prolonged periods which could be facilitated by association with the ER resident chaperone calnexin. The mutants were found to be aggregation prone and capable of eliciting ER stress. Inhibition studies suggested that these mutants are degraded partially by the proteasomal pathway. Further, the degradation of VLDLR wild type and a mutant were delayed in CRISPR/Cas9 edited SEL1L knock-out cells which could be reversed by exogenous expression of SEL1L.

Conclusions: ER retention of VLDLR mutants involves binding to calnexin, elevated ER stress, and delayed degradation which is dependent on SEL1L. Since LDLR family members share common structural domains, common mechanisms may be involved in their processing and trafficking (31M254).

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P04.31C

Biallelic homozygous mutation of *HSPG2* in a patient with dysssegmental dysplasia, Rolland-Desbuquois type

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Dyssegmental dysplasia (DD) is a rare autosomal recessive skeletal disorder characterized by congenital short limbed dwarfism. Based on clinical features, it is classified into two types; the severe, lethal Silverman-Handmaker type (DDSH) and milder form Rolland-Desbuquois type (DDRD). Among the molecularly confirmed DD patients, most are DDSD type. There have been no reports in the patients with molecularly confirmed DDRD. Here, we report a case with homozygous biallelic mutation in HSPG2, diagnosed as DDRD based on clinical features in neonatal period. The proband was 15-years-old boy. He was the first child of non-consanguineous and healthy parents. Physical examination revealed cleft lip palate, shortening of limbs, clubfoot, flexion contractures of bilateral knees and elbows, right inguinal hernia, and narrow chest. X-rays showed small thorax and vertebral body size difference, dumbbell-shaped tubular bones. Based on the radiological investigation, he was diagnosed as DDRD. Now, he is 15 years old, with the height 120 cm (-7.5 SD), weight 27.6 kg (-2.8 SD), and had short distance walkable. To confirm the diagnosis molecularly, we performed Mendelian exome using the TruSight One Sequencing Panel (Illumina, Inc., San Diego, CA, USA). Captured DNA was sequenced on a MiSeq platform (Illumina) with 151 bp paired-end reads. Targeted resequencing and Sanger sequencing identified a novel biallelic homozygous variant c.9970G>A, p.G3324R in HSPG2. This is a first case report with molecularly confirmed DDRD. These results suggest genotype-phenotype correlation in the HSPG2 related disorders.

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P04.32D

Congenital anonychia and uncombable hair syndrome: co-inheritance of homozygous mutations in *RSPO4* and *PADI3*

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Ectodermal dysplasia comprises a heterogeneous group of genetic disorders defined by developmental defects in ectoderm-derived tissues. The great heterogeneity of the symptoms is often an obstacle for the identification of the causative gene, although the use of next generation sequencing has brought new insights. Here, we investigated a 4-year-old Kuwaiti boy showing both congenital anonychia and uncombable hair syndrome. Through whole exome sequencing (WES) we identified mutations in two separate genes, demonstrating that the patient's phenotype comes from the overlap of two autosomal recessive disorders. With regards to anonychia, we identified a previously known homozygous splice-site mutation in *RSPO4*. The encoded protein, R-spondin, is expressed in nail mesenchyme and is an activator of the Wnt/β-catenin pathway. For the hair abnormality, we found a novel homozygous missense mutation in *PADI3*. This gene encodes for peptidylarginine deiminases 3 (PADI3), which is involved in deiminating trichohyalin in the hair follicle, contributing to its structure.All mutations were verified by Sanger sequencing. Furthermore, the *PADI3* mutation was investigated through immunoblotting and immunofluorescence in a keratinocyte cell line, showing both lower expression and formation of aggregates for the mutant protein compared to wild-type. In conclusion, our case highlights the value of WES in identifying co-inheritance of two distinct conditions in consanguineous pedigrees, giving rise to an ectodermal dysplasia phenotype.

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P04.33A

A second patient with Classical Ehlers-Danlos Syndrome (cEDS) and congenital hip dislocation caused by the pathogenic variant *COL1A1* c. 934C>T, p.Arg312Cys

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We present an adult woman with cEDS, bilateral hip dislocation and obstetric anesthetic complications. The patient had generalized joint hypermobility, childhood skin fragility and shoulder dislocations. Her bilateral congenital hip dislocations were treated with Plavik harness. She was diagnosed with cEDS at age 15 following evaluation with multiple affected family members. There was an extensive family history of cEDS features in 7 individuals over three generations spanning in age from 7 to 80 years, none were known to have had bone fragility or vascular complications. The patient had a normal echocardiogram during pregnancy. The patient presented with severe post-partum orthostatic headache after failed epidural anesthesia, during which there was unexpected dural puncture. She recovered after epidural blood patch treatment, following unsuccessful sphenopalatine ganglion block. Sequencing of a large panel of EDS genes, identified the *COL1A1* variant c. 934C>T, p.Arg312Cys. This variant has been reported with a classical EDS like phenotype. The child of patient 1 reported in Malfait et al. (2007), had congenital hip dislocation (1). The variant is mentioned in the 2017 EDS nosology for both cEDS and vascular type EDS (2). Whilst three patients with this same pathogenic variant have been reported to have had a vascular complication, a recent report of a large family was more reassuring (3). We present further cumulative phenotypic data on a large family with this genotype.

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P04.34B

Molecular genetic analysis of Ehlers-Danlos Syndrome in Northwestern region of Russia

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Introduction: Ehlers-Danlos Syndrome (EDS) is a heterogeneous group of connective tissue disorder, caused by metabolic imbalance of collagen, the structure and function of myomatrix also the synthesis of proteoglycans. According to a new international classification of EDS (2017), now it is classified in 13 different types and it is associated with mutations in 19 genes. The exception is the hypermobility type EDS (hEDS) the diagnosis of which is based only on clinical criteria.

Materials and Methods: 12 patients with hEDS were examined. NGS was applied to 10 patients with hEDS and 19 genes associated with EDS were analyzed (*ADAMTS2, B3GALT6, B4GALT7, C1R, C1S, CHST14, COL1A1, COL1A2, COL12A1, COL3A1, COL5A1, DSE, FLNA, FKBP14, PLOD1, PRDM5, SLC39A13, TNXB, ZNF469*). Additionally, direct PCR method was performed to identify del30kb in the *TNXB* for two patients.

Results: We identified a heterozygous mutation c.2818G>A in one patient in the *ADAMTS2* gene with uncertain clinical significance. Mutations in the *ADAMTS2* are associated with autosomal recessive, dEDS. In addition, we detected one heterozygous mutation c.3023C>T with uncertain clinical significance in another patient in the *COL5A1* gene which is associated with cEDS. A heterozygous del30kb in the *TNXB* was identified by direct PCR method in two patients.

Conclusions: Our results indicate that heterozygous mutations in genes associated with different types of EDS can be the cause of hEDS, which indicates genetic heterogeneity of this pathology and needs further research.

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P04.35C

Dual diagnosis of Ellis-van Creveld syndrome and hearing loss in a consanguineous family

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Multilocus analysis of rare or genetically heterogeneous diseases is a distinct advantage of next generation sequencing over conventional single-gene investigations.

We described a female proband from a large consanguineous Iranian family who manifests postlingual, progressive, moderate hearing loss in addition to a disproportionate short-limb dwarfism with distalward shortening of extremities and postaxial hexadactyly. Her brother as well as other members of the family also presented disproportionate short stature and anomalies of the limbs.

Next generation sequencing with a customized skeletal dysplasia panel containing over 370 genes and subsequent bioinformatics analysis disclosed two homozygous mutations in *EVC2* (c.2653C>T; p.Arg885*) and *COL11A2* (c.966dup; p.Thr323His*fs**19), respectively. Sanger sequencing showed that both parents were heterozygous carriers for both *EVC2* and *COL11A2* mutations and the sister was a heterozygous carrier for the *COL11A2* mutation but wild type at the *EVC2* mutation position.

This study highlights a dual molecular diagnosis in a patient with a blending of two distinct phenotypes and illustrates the advantage and importance of this staple technology to facilitate rapid and comprehensive genetic dissection of a heterogeneous phenotype. The differentiation between phenotypic expansion of a genetic disorder and a blended phenotype that is due to more than one distinct genetic aberration is essential in order to reduce the diagnostic odyssey endured by patients.

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P04.37A

Application of a machine learning approach to identify miRNA fingerprint signatures in recessive dystrophic epidermolysis bullosa

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Recessive dystrophic epidermolysis bullosa (RDEB) represents one of the most severe subforms of a rare genodermatosis and is caused by mutations within the *COL7A1* gene. Absent or dysfunctional type VII collagen impedes the structural integrity of the molecular link between epidermis and underlying dermis, which manifests in severe

blister formation sublamina densa and erosions of skin and mucous membranes. RDEB patients suffer from chronically impaired wound healing and an extraordinary high risk of developing a particularly aggressive form of squamous cell carcinoma (SCC). So far there is only very limited data available on non-coding RNA events in RDEB cancer progression.

In order to widen the therapeutic spectrum for RDEB cancer patients we aim to improve our understanding on the impact of differentially expressed small non-coding miRNAs on disease progression and to explore their potential as biomarkers. Therefore, Illumina miRNA-seq was performed on RDEB-SCC, UV-SCC, RDEB and nonEB keratinocytes derived from skin biopsies. Fingerprint miRNAs able to discriminate RDEB-SCCs from the other experimental groups were derived applying a neural network machine learning approach. Specific self-organizing maps were trained on our miRNA-seq data sets and co-expression modules of miRNAs with differential expression profiles extracted. In a guilt-by-association assessment based on microarray expression data, we could demonstrate an enrichment of target mRNAs with strong correlation to signature miRNAs, in hallmark signatures like epithelial-to-mesenchymal transition, which is related to the observed aggressive phenotype in RDEB-SCCs.

Eventually, our set of identified fingerprint miRNAs may provide the foundation for the identification of biomarkers and drugable targets.

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P04.38B
Major player in miRNA processing appear altered in recessive dystrophic epidermolysis bullosa squamous cell carcinoma

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Recessive dystrophic epidermolysis bullosa (RDEB) is characterized by loss-of-function mutations in the *COL7A1* gene, resulting in impaired or absent type VII collagen protein, a major anchoring molecule at the dermal-epidermal junction. Clinically, patients suffer from extraordinary sensitivity of the skin to mechanic friction or trauma and present with an increased risk of infections. One major concern in this patient population relates to the high incidence of developing a particularly aggressive form of squamous cell carcinomas (SCCs), resulting in a highly increased mortality due to frequent metastatic spreading. Recent studies have demonstrated a significantly deregulated miRNome in RDEB-SCC *in vitro*. In order to investigate the cause of the observed altered miRNA abundance, we analyzed expression levels of major components of the miRNA biogenesis pathway via microarray, sqRT-PCR and Western blot. Sanger sequencing of mutational hotspots was performed for major components of the miRNA processing machinery. Cultured primary skin cells derived from patient SCCs and keratinocytes and healthy controls served as experimental groups.

We found significantly increased RNA and protein expression levels of DROSHA in SCC cells as compared to healthy controls, without indication of mutagenic events in reported hotspots. Karyotyping of RDEB SCC cells confirmed chromosomal abberations in regions of interest concerning DROSHA.

A dysregulation of canonical miRNA biogenesis is associated with many diseases, particularly cancer, and could prove instrumental in further deepening our understanding of triggers and promoters of SCC in RDEB.

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P04.39C
Correction of type XVII collagen using spliceosome-mediated RNA *trans*-splicing

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Distinct subtypes of junctional EB (JEB) are caused by mutations within the *COL17A1* gene, encoding type XVII collagen, which leads to fragility of the skin and blister formation within the lamina lucida upon minor friction. So far, there is no causal therapy available for this EB subtype. SMaRT is a post-transcriptional therapeutic approach, which uses the cellular splicing machinery to replace mutation-bearing exons. The aim of this study was to apply SMaRT therapy for the correction of any mutation downstream from *COL17A1* exon 33. We engineered a set of RNA *trans*-splicing molecules (RTMs), which bind

COL17A1 pre-mRNA, thereby inducing a *trans*-splicing reaction that results in the generation of a hybrid mRNA, containing RTM-derived and endogenous gene portions. In a minigene-based approach we first studied the impact of various RTM sequence modifications on *trans*-splicing efficiency and specificity. The modifications included binding domain optimization, removal of the *COL17A1* 3'UTR and removal of potential cryptic splice sites. Retroviral transduction of a JEB keratinocyte line harbouring a compound heterozygous mutation (exon 52 and 53) with the most functional RTM (RTM135mSU), coding for the *COL17A1* wild type exons 34-56, led to restoration of type XVII collagen expression at mRNA level and protein level assessed by immunofluorescence. We found that a binding domain covering the intron/exon junction and the removal of splice sites in the RTMs coding region resulted in an increased *trans*-splicing efficiency. Our results indicate that the SMaRT technology is a promising tool for the development of an RNA therapy for JEB patients.

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P04.40D

Seven additional families with spondylocarpotarsal synostosis syndrome with novel biallelic deleterious variants in *FLNB*

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Introduction: Location and/or type of variants in *FLNB* result in a spectrum of osteochondrodysplasias: spondylocarpotarsal synostosis syndrome and Larsen syndrome that are milder and atelosteogenesis I and III, and Boomerang dysplasia that are perinatal lethal. So far, nine bi-allelic loss-of-function variants in *FLNB* are reported to cause spondylocarpotarsal synostosis syndrome in nine families. We aimed to identify pathogenic variants in *FLNB* in ten patients from seven families with spondylocarpotarsal synostosis syndrome.

Material and Methods: As *FLNB* is a large gene with 46 exons, we preferred exome sequencing index patients from all the seven families as this is cost-efficient at our center followed by Sanger validation and segregation analysis.

Results: Clinical features of ten patients (six females and four males) included short stature (9/9), short neck (6/10), pectus carinatum (5/10), facial dysmorphism (2/10) and cleft lip and palate (1/10). Radiological features were fused vertebrae (9/10), carpal fusion (10/10), tarsal fusion (5/5), scoliosis (9/10), lumbar lordosis (2/10) and crowding of ribs (9/10). Seven novel homozygous variants were identified in *FLNB*: c.6317del [p.(Pro2106ArgfsX12)] in patient 1, c.1493del [p.(Glu498GlyfsX4)] in patient 2, c.1243C>T [p.(Arg415X)] in patient 3, c.1204del [p.(Val402-TryfsX88)] in patient 4, c.28G>T [p.(Glu10X)] in patient 5, c.1429delinsCT [p.(Val477Leufs*2)] in patients 6 and 7, and c.1592dup [p.(His532ThrfsX9)] in patients 8, 9 and 10.

Conclusion: Our work demonstrates that spondylocarpotarsal synostosis syndrome has a unique pattern of anomalous vertebral segmentation and all the reported patients have truncating variants in *FLNB*. The study is funded by Indian Council of Medical Research.

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P04.42B

Novel clinical features in frontometaphyseal dysplasia 2 caused by a recurrent mutation in MAP3K7

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Frontometaphyseal dysplasia 2 (FMD2) is a skeletal dysplasia with supraorbital hyperostosis combined with undermodeling of the bones, joint contractures and some extraskeletal features. It is caused by heterozygous mutations in *MAP3K7*, encoding the Mitogen-Activated Protein Kinase 7. MAP3K7 is activated by TGF-β and plays an important role in osteogenesis. Less than 20 patients with FMD2 and *MAP3K7* mutations have been described thus far. Three out of four patients harbor a recurrent missense

mutation, NM_003188.3: c.1454C>T, p.(Pro485Leu), which leads to a more severe phenotype than mutations in other domains. Here we describe an additional patient with FMD2 caused by the recurrent c.1454C>T *MAP3K7* mutation, identified as a *de novo* variant by whole-genome sequencing. The 17-year-old boy has the characteristic skeletal and facial features of FMD2. However, several novel features were also observed, including multiple hemangiomas, hand and foot abnormalities, growth retardation, spina bifida, Sprengel deformity, Chiari malformation and ocular manifestations. He also showed keloid scars but, in contrast to other patients harboring the same mutation, he does not have intellectual disability. This report expands the clinical spectrum of FMD2 caused by the recurrent c.1454C>T, p.(Pro485Leu) mutation in *MAP3K7*.

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P04.44D

MicroRNA profiling in human neural crest cells and integration of GWAS data suggest involvement of miRNA149 in craniofacial disease

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GWAS for nonsyndromic cleft lip with/without cleft palate (nsCL/P) have identified 40 risk loci for this human craniofacial malformation. The vast majority of these associated loci map to non-coding genomic regions. One possible mechanism by which variants at these loci might exert their regulatory effect is the control of microRNA (miRNA) expression and miRNA-mediated gene regulation, in disease-relevant tissue. In the present study, we sought to identify candidate miRNAs in human neural crest cells (hNCCs), an early precursor cell population of facial tissue, and integrate GWAS data to identify potential susceptibility miRNA candidates that might be involved in nsCL/P.

MiRNA profiling was performed in four independent hNCC samples, previously generated from induced pluripotent stem cells, using the Affymetrix miRNA 4.0 array platform. After stringent filtering, 152 miRNAs were identified as expressed in hNCCs, none of which mapped to the known nsCL/P loci. However, positional integration of GWAS summary statistics revealed 25 variants (at P-value <0.01) that were located within 1kb of a candidate miRNA. Association of one variant, a low-frequency variant at chr. 2q37.3 close to miRNA-149, was confirmed in a replication analysis of an independent nsCL/P cohort. Target gene prediction with miRWalk2.0 and literature research revealed that miRNA149 likely binds known nsCL/P candidate genes such as *FGFR1*, *BMP9* and *RUNX2*, and has been shown to differentially interact with MTHFR upon folate deficiency. Although further functional characterization is required and currently ongoing, our study suggests that miRNA-149 might be involved as regulatory mechanism in facial development.

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P04.45A

Genetic analysis of genodermatoses in domestic animals

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Introduction: Spontaneous mutants in domestic animal species are valuable models to study heritable human

disorders. Purebred animals are kept in closed populations necessitating a certain degree of inbreeding, which favors the expression of recessive alleles. Due to the unique population structure of purebred animals, identification of disease causing genetic variants is often more straightforward than in humans.

Materials and Methods: Genetic mapping and whole genome sequencing approaches were used.

Results: We identified candidate causative variants for several genodermatoses including variants in genes that had not previously been associated with disease phenotypes in humans. As an example, we discovered genetic variants in *SUV39H2* in dogs with hereditary nasal parakeratosis, which revealed a role for *SUV39H2* in keratinocyte differentiation.

Selected domestic animal models for human genodermatoses

Gene	Phenotype	Species	Human disorder (MIM#)
ASPRV1	ichthyosis	dog	?
EDA	X-linked hypohidrotic ectodermal dysplasia	dog, cattle	305100
FAM83G	hereditary footpad hyperkeratosis	dog	palmoplantar keratoderma and exuberant scalp hair
IKBKG	incontinentia pigmenti	horse	300291
MBTPS2	brindle 1	horse	300918, 308205, 308800
NSDHL	congenital cornification disorder	dog	308050, 300831
OCA2	oculocutaneous albinism, type 2	dog	203200, 227220
ST14	naked foal syndrome	horse	602400
SUV39H2	hereditary nasal parakeratosis	dog	?
TSR2	streaked hairlessness	cattle	?

Conclusions: The study provides new candidate genes for genodermatoses in human and veterinary medicine and a better understanding of the genotype-phenotype correlation.

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P04.46B

Novel case with a double "apparently" balanced rearrangement disrupting *EXT1* in a patient with hereditary multiple exostoses

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Hereditary multiple exostoses (HME) is an autosomal dominant skeletal disorder characterized by the development of multiple, circumscript, occasionally painful and usually symmetric bony protuberances called osteochondromas. HME is caused by *EXT1* and *EXT2* loss of function mutations. Most pathogenic mutations are nonsense followed by missense mutations and deletions. We report on a patient with a rare and complex genotype resulting in a classical HME phenotype.

Mutations in *EXT1* and *EXT2* were excluded by Sanger sequencing. The patient was subsequently referred for karyotype and array-CGH analyses. Results obtained were validated with FISH and qRT-PCR and parental studies determined the mode of inheritance.

Chromosomal analysis revealed a *de novo* "apparently" double balanced rearrangement: a balanced translocation between chromosomes 2 and 3 at breakpoints 2q22 and 3q13.2 and a pericentric 8p23.1q24.1 inversion both of which were confirmed by FISH analysis. Subsequently, array-CGH analysis revealed a novel heterozygous deletion within the *EXT1* gene at the inversion breakpoints, rendering the inversion as unbalanced. The inheritance mode as well as the size of the deletion was further investigated by qRT-PCR and the deletion was characterized as a *de novo* 3.1kb deletion removing exon 10. The inversion in combination with the 8p23.1 deletion most likely abolishes the transcription of *EXT1* downstream of exon 10 hence resulting in a truncated protein.

To conclude, a rare and novel pathogenic cause of HME is presented in this study, highlighting the importance of additional comprehensive cytogenetic investigation when *EXT1* and *EXT2* mutation analysis is negative.

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P04.47C

An insertion mutation in *HOXC13* underlies pure hair and nail ectodermal dysplasia with lacrimal duct obstruction

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Ectodermal dysplasias (EDs) encompass a large group of clinically and genetically heterogeneous hereditary disorders that are defined by abnormal development of at least two ectodermal structures, which include but are not limited to hair, nails, teeth and sweat glands. A rare form of autosomal recessive ED that is usually characterized by hypotrichosis and nail dystrophy only is known as pure hair and nail ectodermal dysplasia (PHNED). To date, mutations in *KRT85*, *KRT74*, and *HOXC13* have been reported in patients and families of different ethnic origin with PHNED. Here, we studied two sisters from a consanguineous Iranian marriage who were not only affected by hypotrichosis and nail dysplasia but also lacrimal duct obstruction (LDO). Homozygosity mapping and GeneDistiller analysis suggested linkage of the disease to chromosome 12q13.13. In this region, *KRT85*, *KRT74*, and *HOXC13* were the most plausible candidate genes. Sanger sequencing of *HOXC13* revealed a hitherto undescribed homozygous 28 bp insertion mutation (c.837_838insACTTGCGGCTAGCAAGTTCATCACCAAA; p. A280Tfs*4) in exon 2 in both affected children that was present in the heterozygous state in the parents.

LDO has not previously been described in association with PHNED although this symptom has been frequently observed in other types of ED. Therefore, LDO might have been neglected or underdiagnosed in earlier reports of PHNED. The clinical and molecular genetic findings in the family expand the phenotypic and mutation spectrum of PHNED and suggest that LDO should be examined for when clinically evaluating individuals and families with this rare variant of ED.

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P04.48D

Italian validation of the functional difficulties questionnaire (FDQ-9) and its correlation with major determinants of quality of life in adults with hypermobile Ehlers-Danlos syndrome/hypermobility spectrum disorders

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The 2017 EDS International Classification defined the new criteria for hypermobile Ehlers-Danlos syndrome (hEDS), which is now considered one end of a continuous spectrum originating from isolated, non-syndromic joint hypermobility (JH) and passing through hypermobility spectrum disorders (HSD). Preliminary data indicate a link between JH and neurodevelopmental disorders, and the strongest evidence is the non-causal association with developmental coordination disorder (DCD) in children. Assessing DCD in adults is difficult and the recently described functional difficulties questionnaire 9 (FDQ-9) is one of the few available tools. The aims of this study were: (i) to validate FDQ-9 in Italian and to normalize its values in 230 Italian non-clinical patients; and (i) to explore the relationship of FDQ-9 with the brief pain inventory, composite autonomic symptom score 31, multidimensional fatigue inventory, ADHD self-report version 1.1, and the SF-36 for quality of life in 105 Italian adults with hEDS/HSD. Validity of FDQ-9 was assessed by the Pearson test in 10 bilingual individuals and 5 bilingual hEDS/HSD patients who completed the questionnaire in English and Italian. In the hEDS/HSD group, 67% patients had a value of FDQ-9 above the cut-off and, therefore, a high probability of DCD in their developmental age. Multivariate analysis was carried out comparing FDQ-9 values with features of pain, fatigue, autonomic dysfunction, ADHD and quality of life, and demonstrated an

influence of a past history of coordination troubles on chronic symptoms in adults with hEDS/HSD. Our preliminary data open wider management and therapeutic perspectives for coordination troubles in hypermobile individuals.

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P04.49A

Detection of mosaic Copy-Number Variations from Whole-Exome Sequencing in mosaic pigmentation disorders using XHMM and a custom SNP approach

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Whole exome sequencing (WES) is as a powerful tool for deciphering the genetic basis of developmental disorder, either single nucleotides variants (SNV), indels, or recently copy number variants (CNV). In mosaic development disorders involving the skin, it has allowed detection of post-zygotic mutations (mSNV) in various genes, but detection of mosaic CNV (mCNV) still relies on conventional cytogenetic studies, such as array-CGH. We sought to develop an all-in-one strategy for patients with mosaic disorders, using trio-WES (lesional skin versus parent's blood). We combined a SNV detection pipeline with a CNV detection approach, based on both a read-depth approach and a custom SNP-based approach.

We performed WES in 74 patients with cutaneous mosaic disorders. We detected 6 mCNV, all in a subgroup of 21 patients with hypomelanosis of Ito without known pathogenic SNV: 3 mosaic trisomy (chromosomes 7, 12, 15) and 3 smaller mCNV. Blood and skin karyotypes were previously negative, due either to the absence of mosaic cells in blood or to their elimination from cultured fibroblasts. For the 3 mosaic trisomies, SNP inheritance and b-allele frequency provided information on the parental origin of extra chromosomes and clues to understand the underlying mechanism. We have confirmed that chromosomal mosaicism is associated with mosaic pigmentation disorders (6/21, 29% in our cohort). An appropriate fresh tissue sample is essential. Our combined approach showed a good efficiency to detect both (m)SNV and (m)CNV in a single one-step assay, doubling our diagnostic rate, and offering new perspectives in the study of mosaic developmental disorder.

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P04.50B

Results of diagnostics of ichthyoses and epidermolysis bullosa using dedicated next generation sequencing panel

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Introduction: Ichthyoses (I) and epidermolysis bullosa (EB) are rare, monogenic skin comprising several dozens of clinical entities, caused by mutations in over 36 and 21 genes, in I and EB, respectively. Low incidence, genetic diversity, overlapping phenotypes and age-dependent disease course negatively influence the detection rate in traditional diagnostic procedure based on phenotype to genotype approach. The aim of the study was to elaborate the cost effective genodermatoses-dedicated next generation sequencing (NGS) panel.

Materials and Methods: We enrolled 103 and 8 patients presenting clinical symptoms of I and EB, respectively. A self-designed panel of 86 genes (Roche NimbleGen SeqCap EZ System) for library preparation and MiSeq sequencer (Illumina) were used, followed by Sanger sequencing/ MLPA for mutations verification.

Results: We identified full genotype in 86/103 (83%) of I and 7/8 (87%) of EB patients. In 12/103 (12%) of I patients we didn't detect any mutation, while in 6 (5 I and 1 EB) we found mutation in one allele only. Overall, we detected mutations in 19 distinct genes. Furthermore, in 10 patients, in addition to their primary disease-causing mutations, harbored also another possibly pathogenic mutation in one allele of the other gene, including semi-dominant mutations in FLG.

Conclusions: In conclusion, our panel proved to be an efficient and sensitive first-line diagnostic tool. Our data provide further information regarding molecular epidemiology of I and EB and also focus on the presence of secondary mutations, which have important impact on genetic counseling and, presumably, may affect therapy. Supported by grant NCN 2014/13/D/NZ5/03304

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P04.52D

Ichthyosis prematurity syndrome identified for the first time in the Spanish population. Unprecedented molecular characterization of a *FATP4* splicing variant in humans

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Introduction: Ichthyosis prematurity syndrome (IPS) is a rare syndromic form of autosomic recessive ichthyosis caused by mutations in *FATP4*. To date, three different *FATP4* splice site mutations have been associated with IPS,

but none of them has been yet characterized at RNA level in humans.

Materials and Methods: Two 26 and 27 years-old Spanish siblings who presented with congenital ichthyosis clinical manifestations, and tested negative for autosomal recessive congenital ichthyosis (ARCI) related genes, were sent to our service. Clinical features were carefully assessed and genetic analysis was performed in both patients and their parents. *In silico* predictions of mutational effects and further characterization by RNA study was performed for one putative splicing variant identified.

Results: Two novel *FATP4* mutations were found in both patients: one frameshift variant, c.1322dup, p. Gly442Argfs*2 and one intronic substitution, c.988-19A>G. Parents were heterozygous for each of the variants. Molecular characterization of c.988-19A>G showed that this variant creates one aberrant transcript that lacks the first 45bp of exon 8, which encodes the end of the protein ATP/AMP motif, and it also leads to a deregulation of naturally occurring isoforms. *In silico* analyses predicted the variant to alter the recognition sites for splicing regulatory proteins.

Conclusions: This study describes, for the first time, two cases of genetically diagnosed IPS in the Spanish population, adding new mutations to the current list of *FATP4* pathogenic variants. It also characterizes the non canonical splice-site c.988-19A>G mutation, being the first splicing study in *FATP4* in humans.

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P04.54B
Novel mutations and clinical variability in Van der Woude Syndrome

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Van der Woude Syndrome (OMIM 119300) is an autosomal dominant clinical condition characterized by cleft palate, cleft lip, and lower lip pits. vWS is the most common cause of syndromic cleft lip-palate, and also 2% of all cleft lip and palate cases. vWS presents with variable phenotypic features and high penetrance. Interferon Regulatory Factor 6 (*IRF6*) gene mutations have been reported as the cause of vWS. The 3th and 4th exons of the gene encode DNA binding domain; 7,8, and 9th exons encode protein binding domain. More than 300 *IRF6* mutations have been reported as the cause of vWS syndrome. Mutations are mostly clustered in exons encoding functional domains. Mutations in the *IRF6* gene are also associated with the Popliteal Pterygium Syndrome(OMIM 119500), which has similar orofacial features with vWS; however, it also presents with popliteal webs, syndactyly, and genital anomalies. Here we report, 43 patients with vWS at 6 different families and two of them have novel mutations, which have not reported before. A novel c.841-2A>C mutation was identified in family A and a novel c.881T>A mutation was identified in family B. These mutations will provide a better understanding of the phenotypic effect of exon 7 mutations. Due to variable expression, the same mutation can lead to different clinical manifestations. However, different mutations in the same genes can also be presenting with several phenotypes. For this reason, the clinical effect of new mutations help us for better understanding the causes of the disease. It contributes to the genetic counselling.

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P04.55C
Do homozygous mutations in the upstream of Ig-like C2-type 2 domain of FGFR1 result in isolated ectrodactyly?

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Introduction: Ectrodactyly, also known as split hand/foot malformation, is seen in 1 of 8,500-25,000 newborn as isolated or part of a syndrome. There are various types of ectrodactyly that are inherited with autosomal recessive, dominant and X linked manner. Ectrodactyly is also a component of Hartsfield syndrome resulting in homozygous or heterozygous mutations in Ig-like C2-type 2 domain (conserved) of FGFR1 gene. Our purpose for presenting this work is to discuss homozygous mutations in the upstream region of FGFR1 gene conserved region may be a cause of isolated ectrodactyly.

Materials and Methods: We performed next-generation sequencing (NGS) panel (Trusight one sequencing panel) for a 2 years old female with left-hand ectrodactyly. Neuromotor and growth development of the patient were normal and no abnormality was observed in cranial MRI and echocardiography. All laboratory tests including biochemical and metabolic screenings were also normal.

Results: A homozygous mutation (c.386A>C; p. Asp129Ala) was detected with NGS panel in the upstream of Ig-like C2-type 2 domain of FGFR1 gene. p.Asp129Ala hasn't been reported before and insilico tools predicted the mutation as deleterious. The mutation is detected as

heterozygous in unaffected parents and expression analysis currently continues.

Conclusion: Hartsfield syndrome, characterized by ectrodactyly and holoprosencephaly, is the result of FGFR1 gene conserved domain mutations. Up to now, FGFR1 mutations have not been reported in patients with isolated ectrodactyly. This report is unique in terms of showing that homozygous mutations in upstream of Ig-like C2-type 2 domain of FGFR1 gene are related with isolated ectrodactyly.

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P04.57A

Further delineation of the linkeropathy syndrome due to glucuronyltransferase I-deficiency in a family with *B3GAT3* compound heterozygosity for two novel mutations revealed by whole exome sequencing

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Linkeropathies are a group of heterogeneous syndromes along a spectrum of skeletal and connective tissue disorders (CTDs) with the full disease range yet to be defined. Linkeropathy genes encode for enzymes branching glycosaminoglycan chains onto proteoglycans via a common tetrasaccharide linker; *XYLT1* and *XYLT2* encode for xylosyltransferases, *B4GALT7* and *B3GALT6* for galactosyltransferases, and *B3GAT3* for a glucuronyltransferase. *XYLT1* and *XYLT2* are associated respectively with Desbuquois dysplasia type 2 and with spondylo-ocular syndrome, *B4GALT7* and *B3GALT6* with spondylodysplastic Ehlers-Danlos syndrome (spEDS). For *B3GAT3*, 23 patients, all but one from 9 consanguineous families with homozygous missense variants, have been described ranging in phenotypic severity from mild to severe and resembling Larsen-, Antley-Bixler-, Shprintzen-Goldberg-, and Geroderma osteodysplastica-like syndromes. Here, we report on a 16-year-old girl with a clinical suspicion of spEDS. She was born to non-consanguineous parents and presented with facial dysmorphism (dolichocephaly, prominent forehead, enophthalmos, midface hypoplasia, micrognathia, low-set ears), short stature, muscle hypotonia, severe kyphoscoliosis, joint laxity with recurrent dislocations, pectus carinatum, bilateral radio-ulnar synostosis, atlanto-occipital instability, and bilateral pes planovalgus. Medical history included severe low bone density, congenital hip dislocation, atrial septal defect, and anterior ectopic anus. Trio analysis by WES revealed compound heterozygosity for two novel missense mutations in *B3GAT3*, thus expanding its allelic repertoire. We provide a comparative overview of the phenotypic features of linkeropathies headlining the extended phenotypic range of *B3GAT3* mutations that overlaps with skeletal dysplasias and other CTDs including EDS, hence offering future perspectives for EDS nosology and clinical research in this field.

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P04.58B

Higher diagnostic yield can be achieved by using multigene NGS panel in testing patients with Marfan-related disorders

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Introduction: Marfan syndrome is a life threatening condition with estimated prevalence of 1:5,000-1:10,000. All cases appear to be due to heterozygous mutation in *FBN1* gene. However, there are other heritable conditions with partially overlapping phenotypes caused by other genetic defects. Here, we compare the diagnostic yield of using NGS multigene panel with *FBN1*-only testing, in 34 patients.

Methods: Targeted NGS was applied to analyze 34 samples from individuals with clinical presentation of Marfan-related disorders. Twenty samples were analyzed for *FBN1* gene only, and 14 samples were analyzed for a panel of 14 genes named as "Marfan, Aneurysm and Related Disorders". Target regions captured with Nimblegen chip, followed by NGS on Illumina platform. Candidate variants were interpreted according to ACMG-guideline for variant interpretation 2015.

Results: In 10 samples, out of 20 samples, that were analyzed for *FBN1* gene only, a strong candidate causative variant with pathogenic, likely pathogenic or VUS classification were detected. Other 10 samples end up with a negative result. On the other hand, all 14 samples tested for the panel of 14 genes, resulted strong candidate causative variants. 9 out of 14 carried a causative variant in *FBN1*

gene (64%), while 5 samples had possible causative variants in five other genes. Missense variants were the most common causative variants and in total 7 novel variants were also identified.

Conclusion: This study showed using NGS panel of genes associated with Marfan-related disorders, gives a higher diagnostic yield for such patients. Nearly 30% found in this study were novel variants.

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P04.61A

Two novel probands with Myhre syndrome identified through whole exome sequencing

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Introduction: Myhre syndrome (MYHRS) (MIM 139210) is a rare autosomal dominant multisystem connective tissue disorder, characterized by mental retardation, cardiopathy, skeletal abnormalities, short stature, scleroderma and laryngeal stenosis. So far, all reported cases were due to *de novo* gain-of-function missense mutations in *SMAD4*, encoding the SMAD protein commonly required for both transforming growth factor-beta and bone morphogenic proteins signal transduction.

Materials and Methods: We report on two adult probands with MYHRS identified through whole exome sequencing and performed transmission electron microscopy (TEM) on a skin biopsy.

Results: The first proband presented with a congenital heart defect and vertebral anomalies, but normal skin, stature and intelligence. Later-on, she developed severe laryngeal stenosis. She has two similarly affected children. The second proband presented with visual impairment following lensectomy in childhood, short stature, brachydactyly, stiff skin and decreased peripheral sensitivity. Both patients harbor the recurrent heterozygous c.1486C>T (p.Arg496Cys) mutation in *SMAD4* (NM_005359). TEM of the dermis of the second proband shows dens collagen and irregular elastin cores with globular deposits and almost absent surrounding microfibrils.

Conclusions: We report on two novel probands with MYHRS. To our knowledge our data represent the first familial case of MYHRS and further widens the clinical spectrum of the disorder. TEM analysis implicates both collagen and elastic fiber anomalies and may shed novel insights on the relation between growth factor signaling and extracellular matrix homeostasis.

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P04.62B

CALMPlex: a multi-gene panel for variant detection in patients with Phakomatoses and overlapping disorders

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Phakomatoses, characterized by pigmentary manifestations, are phenotypically overlapping disorders often difficult to distinguish only on the basis of clinic in early childhood. Nevertheless, a proper diagnosis is essential for an appropriate clinical management. We developed CALMPlex, a targeted NGS platform for molecular diagnosis of Phakomatoses. It comprises 70 disease-causing genes, including genes responsible for Phakomatoses, *RNF135* and *SUZ12* as *NF1* flanking genes involved in 17q11 microdeletion, *SPRED2* and *SPRED3* as *SPRED1* homologous genes and all the genes of RASopathies. It also includes 25 candidate genes identified as direct interactors of the selected disease genes with different bioinformatics tools (i.e. STRING, GeneMANIA). We enrolled 149 children (0-18 years) with clinical diagnosis of Phakomatoses. Additional 13 samples with already known mutations were used as training set to validate CALMPlex. 10/149 patients were clinically diagnosed as NF1 not confirmed by Sanger sequencing at RNA analysis. CALMPlex detected all disease-causing mutations

in the training set and a NF1 mutation in 100% (10/10) of molecularly unresolved NF1 cases. In the other 139 cases, CALMPlex identified the molecular defect in 57%(80/139) of them: 77 had mutations in genes fitting the clinical diagnosis (*NF1*, *NF2*, *SPRED1*, *PTPN11*, *KIT*, *TSC1*, *TSC2, LZTR1 and PPP1CB*), while 3 had mutations in an unexpected gene respect to initial clinical suspicion. Pathogenic variants in candidate genes have also been detected in some of patients remained undiagnosed but a further characterization is necessary. CALMPlex is a useful first-tier test for genetic evaluation of these phenotypically overlapping conditions especially when only pigmentary manifestations occur.

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P04.63C

Genetic diagnosis of bone mineralisation disorders with Next Generation Sequencing and definition of five novel pathogenic variations

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Introduction: Bone mineralisation disorders are a very heterogenious group of bone disorders both in terms of clinical manifestations and genetic background. Next Generation Sequencing (NGS) is a powerfull technology allowing analysis of a lot of genes of a number of patients simultaneusly with a low cost in a short time. We aimed to report six novel variations of bone mineralisation disorders-related genes in 6 different patients directed to our department with clinical diagnosis of abnormal bone mineralisation.

Materials and Methods: Genomic DNA samples were isolated from EDTA-blood samples of 11 patients. Libraries were prepared with Osteo-GeneSGKit DensidadOsea, IVD -CE kit according to instructions of manufacturer's. Fastq generation was performed by MiSeq Reporter (v2.5.1; Illumina Inc.) Genomize Seq. Platform was used for variant calling and filtering. Varsome was used for in silico analysis of variants and ClinVar and HGMD databases were accepted as refference for known variant interpretation,

Results: We defined 5 novel and 4 known mutations in 9 (4 variants in COL1A1, 2 variants in COL1A2, 1 variant in PHEX, 1 variant in TGFB1 and 1 variant in SLC34A1) out of 11 patients (81.81 %).

Conclusion: We suggest that sequencing of the genes associated with bone mineralisation simultaneously with Next Generation Sequencing offers a practical approach to define genetic background of this type of heterogenious diseases.

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Large-scale resequencing study of nsCL/P candidate genes in 1061 nsCL/P cases and 1591 controls

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Non-syndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital malformations and has a multifactorial etiology. To date, a number of common risk variants have been identified for nsCL/P, explaining about 25% of the genetic heritability. We hypothesize that some of the remaining genetic liability is explained by rare dominant *de novo* mutations. In order to identify such rare *de novo* events, we performed whole exome sequencing (WES) in 50 nsCL/P patients and their unaffected parents. The analysis resulted in 33 rare *de novo* events in 33 genes. To find further support for these candidate genes and to strengthen our hypothesis of dominant *de novo* events adding to nsCL/P etiology, the candidate genes were subjected to a multiplex resequencing study with single molecule molecular inversion probes (smMIPs) in a multiethnic case/control sample of Arabian, Mexican and Central European ancestry (n_{cases}=1,061, $n_{controls}$=1,591). The assay

was designed using the standard MIPgen pipeline and was successful for 32 genes. Libraries were sequenced on an Illumina HiSeq2500 2x125bp using Illumina v4 paired-end chemistry. Raw reads were aligned with BWA and variants were called with UnifiedGenotyper. Downstream analysis included filtering for CADD ≥ 15 and MAF ≤ 0.1% followed by a manual inspection of reads and a validation step including segregation analysis. Our preliminary results of the resequencing approach show the presence of further rare variants/rare *de novo* events in our candidate genes in nsCL/P patients. Further results will be presented at the conference.

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P04.66B

Opsismodysplasia - report on long-term follow-up of a previously described and two new Portuguese cases

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Introduction: Opsismodysplasia (OPSM) is a rare autosomal recessive spondyloepimetaphyseal dysplasia caused by biallelic mutations in *INPPL1* gene, which encodes SHIP2, one of the phosphatases that catalyse dephosphorylation at the 5-position of phosphoinositides. OPSMD is characterised by severe pre and post-natal micromelia with extremely short hands and feet, large fontanel, craniofacial dysmorphisms and typical radiographic features such as major delay in bone maturation, platyspondyly, squared metacarpals and metaphyseal cupping.

Case Reports: We report on three unrelated Portuguese patients, two male and one female, with the diagnosis of OPSM based on their clinical, radiographic and molecular findings. Patient 1 was the 6th case described in the literature and is now 25 years, likely one of the oldest known patients alive. From his follow-up we highlight: adult height of 1.05m; spinal surgery for severe scoliosis (4 years); severe atlantoaxial instability; bilateral cryptorchidism; mitral valve prolapse; noninvasive ventilation during sleep (since 15 years); without other significant problems. Patient 2 (5 years) has also typical features while Patient 3 (8 years) has a milder phenotype, in accordance with his genotype: compound heterozygous for c.1497+5G>C and c.1649T>C (p.Phe550Ser) in *INPPL1* gene. Patients 1 and 2 are homozygous for null mutations: c.2719C>T(p.Arg907*) and c.768-769del(p.Glu258Alafs*45), respectively. Neurodevelopmental assessments showed normal cognitive function and marked motor delay.

Discussion: Once thought to be a lethal condition, OPSM turned out to have a wider phenotypic variability, well-illustrated in the description of these 3 patients. Despite the growing number of reported surviving cases, information is lacking on natural history and long-term follow-up.

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P04.67C

Whole exome sequencing in Finnish families identifies new candidate genes for osteoarthritis

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Introduction: Osteoarthritis (OA) is the most common degenerative joint disease characterized by progressive degradation of the joint cartilage. OA is a complex disease that has a strong genetic background with heritability estimations of 39% and 60% for knee and hip OA, respectively. The objective of this study was to identify rare variants predisposing subjects to OA in three Finnish families.

Materials and Methods: Eight subjects from three families with hip and knee OA were studied using whole exome sequencing. We focused on rare exonic variants with predicted pathogenicity and variants located in active promoter or strong enhancer regions. The software tool ANNOVAR was used for functional annotation and minor allele frequencies were obtained from the Exome Aggregation Consortium (ExAC) and Sequencing Initiative Suomi (SISu) database. Expression of identified genes in human bone and cartilage tissue was studied using PCR.

Results: Two rare variants co-segregated with OA in two families. In Family 8 a missense variant was observed in the *OLIG3* gene that encodes a transcription factor known to be associated with rheumatoid arthritis and inflammatory polyarthritis. In Family 12 the observed variant was located in the transcription start site of the *FIP1L1* gene. *FIP1L1* participates in the regulation of polyadenylation. Both *FIP1L1* and *OLIG3* were observed to be expressed in human bone and cartilage tissues.

Conclusion: The exome sequencing revealed novel candidate genes for OA. *OLIG3* and *FIP1L1* may participate in the regulatory events leading to OA.

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Mutation spectrum, genotype-phenotype correlation & digenic inheritance in a large Indian cohort of osteogenesis imperfecta

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Introduction: Osteogenesis Imperfecta (OI) is a clinically and genetically heterogeneous disorder. Till date, pathogenic variants in approximately twenty genes are known to cause OI with mutations in the type 1 collagen genes (*COL1A1* and *COL1A2*) being the most common cause.

Materials and Methods: In our study, we evaluated the clinical features of OI in 50 Indian index patients. Grouping according to phenotypic and radiographic features revealed four individuals with Bruck syndrome, three patients with hypertrophic callus and twenty with extreme bone bowing. The molecular evaluation was done by a small custom designed gene panel or exome sequencing.

Results: In all but two patients, pathogenic variants in known disease genes were detected. We observed a total of 24 novel mutations and 24 known OI mutations, of which several were recurrent. In one patient no relevant mutation was found, and another patient harboured a class III *COL1A1* intronic variant. The percentage of autosomal recessive forms due to mutations in *BMP1, FKBP10*, *LEPRE1*, *SERPINF1*, and *WNT1* was unusually high (48%). Cases with *FKBP10*, *IFITM5*, and *WNT1* mutations could best be distinguished clinically. Most severe forms were due to *IFITM5* and *LEPRE1* mutations, followed by qualitative *COL1A1, SERPINF1*, and *WNT1* mutations. Quantitative *COL1A1* mutations and *COL1A2* mutations had milder effects. In one family we found evidence for a digenic inheritance pattern due to heterozygous variants in *COL1A1* and *COL1A2*.**Conclusions:** The findings in our large cohort demonstrate the clinical utility of gene panel testing for OI.

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P04.69A

Sclerostin and bone: The role of the *SOST* gene in osteoporosis and fragility fractures in Malta

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Introduction: Sclerostin is an important regulator of the bone remodelling cycle acting as an inhibitor of the canonical Wnt signalling pathway, resulting in reduced bone mass. The aim of the study was to identify known or novel *SOST* variants associated with osteoporosis and fracture susceptibility in the Malta Osteoporotic Fracture Study (MOFS).

Materials and Methods: Sanger sequencing of the *SOST* exons and their intronic flanking regions, together with the promoter and 3'untraslated region (UTR) was performed in 200 individuals having normal and low bone mineral density (BMD).

Results: A total of 10 known and 3 novel variants were identified including: rs851055, rs140960915, rs117857467, rs59613373, rs17882143, rs768384322, rs199560099, rs17883310, rs17881550, rs17886183, c.220+134(T>C), c*331(A>G) and c*390(C>A). Preliminary logistic regression with regards to the rs851055 promoter variant (G>A), indicate that homozygosity for the A allele was associated with a protective effect on BMD at the lumbar spine, LS (Age adjusted Odds ratio: 0.1 [95% confidence interval 0.03-0.8]) and total hip, TH (OR: 0.3 [0.1-1.0]). The rs17881550 3' UTR insertion (-/G) also showed a protective effect on LS BMD (OR 0.1 [0.03-0.7]), TH (OR 0.2 [0.04-0.9]), and also with fracture risk (OR 0.2 [0.05-0.9]) in women with the homozygous mutant genotype compared to women with the homozygous wild-type genotype.

Conclusion: Observations suggest that the rs851055 and rs17881550 variants might be affecting transcriptional activation or epigenetic mechanisms resulting in altered Sclerostin function. All variants will be replicated in the entire MOFS collection to determine association with BMD and fracture susceptibility at different anatomical sites.

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P04.70B

A novel recurrent mutation (c.373delC) in HPGD gene is responsible for Pachydermoperiostosis

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Pachydermoperiostosis (primary hypertrophic osteoarthropathy, Touraine-Solente-Gole syndrome, MIM 167100) is a rare genetic disorder characterized by digital clubbing, pachydermia and periostosis. Genetic background of this disease has been recently revealed. Homozygous and compound heterozygous mutations in HPGD gene cause insufficiency of 15-hydroxyprostaglandin dehydrogenase, an enzyme responsible for PGE2 catabolism. Elevated levels of PGE2 were reported in all homozygous patients, thus leading to chronic inflammation process, most strikingly affecting joints.

We recently diagnosed two unrelated patients from non-consanguineous families, age 7 and 23, both with digital clubbing, prominent sweating of hands and feet and joints pain. In older patient pachydermia and excessive sweating was noticeable, however in younger patient the skin is not yet affected. HPGD gene analysis was performed and in both of them the same two truncating mutations were found – c.175_176delCT and c.373delC. Biparental origin of these mutations was confirmed. First mutation (c.175_176delCT) is the most common mutation of HPGD gene in Caucasian population, whereas second mutation (c.373delC) has not been reported previously.

We assume that the c.373delC truncating mutation of HPGD gene, together with the c.175_176delCT mutation, might be the founder mutations in Polish population, responsible for more cases of Pachydermoperiostosis syndrome.

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P04.71C

Polymorphisms in genes involved in the base excision repair (BER) pathway are associated with susceptibility to Paget´s disease of bone

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Introduction: Paget´s disease of bone (PDB) is a chronic bone metabolic disorder. Currently, PDB is the second most frequent bone disorder. PDB is a focal disorder affecting the skeleton segmentally. Its cause is unknown, but it has been hypothesised that somatic mutations could be responsible for the mosaicism described in PDB patients. Therefore, our hypothesis is that defective response to DNA damage may lead to somatic mutations, which in turn increase the risk of PDB.

Materials and Methods: We analysed polymorphisms in DNA repair genes involved in the BER, NER and DSBR pathways in order to evaluate the role of these variants in modulating PDB risk.

Results: We found statistically significant differences in genotypic and allelic distribution for polymorphisms in genes involved in the BER pathway. Our results showed that carrying the allele T of the XRCC1 rs1799782 polymorphism and the allele G of the APEX rs1130409 polymorphism increased the risk of developing PDB.

Conclusions: These polymorphisms could cause a lower DNA repair efficiency and this might lead to local somatic mutations favouring the bone metabolic alterations characteristic of PDB. This is the first report showing an association between polymorphism in genes involved in the BER pathway and PDB.

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P04.72D

Screening a French series of Ehlers-Danlos syndrome patients with periodontal manifestations reveals new variants in *C1R* and *C1S* and refines the clinical features of periodontal Ehlers-Danlos syndrome

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Background : Pathogenic variants in *C1R* and *C1S* were recently discovered as a cause for periodontal Ehlers-Danlos syndrome (pEDS, previously EDS VIII). EDS are connective tissue disorders defined by major criteria: joint laxity and skin alterations. pEDS share several features with vascular EDS (vEDS), like acrogeria, gum fragility and arterial or gastrointestinal ruptures.

Methods : *C1R* and *C1S* were screened by Sanger Sequencing in a series of 20 cases with periodontal manifestations and addressed to the French National Reference Centre for vEDS (Georges Pompidou European Hospital, Paris, France).

Results : We found 3 unrelated cases harboring *C1R* or *C1S* missense variants: p.Tyr302Cys (previously reported) and p.Cys309Phe in *C1R*, p.Cys321Ser in *C1S*. The two last variants affect cysteines in CCP1 domains, like most described pathogenic variants. These affected cases had three characteristic features: early-onset periodontitis with complete tooth loss, easy bruising and pretibial hyperpigmentation. Two were sporadic cases and one had a family history of periodontitis. The negative cases had unusual periodontal manifestations and severe joint hypermobility. We are currently building a database of confirmed pEDS patients to study the role of pathogenic variants in the immune response because of recurrent infections (40% of cases).

Conclusions : We confirmed pEDS is a rare condition with a well-defined phenotype including the three features abovementioned. The clinical diagnosis should lead to genotype *C1R* and *C1S*. The pathophysiology remains to be discovered, including the impact in the immune response system.

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P04.73A

PLS3 deletions lead to severe spinal osteoporosis and disturbed bone matrix mineralization

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Introduction: *PLS3* is one of the genes associated with X-linked osteoporosis in children and pathogenetic variants have thus far been reported in 16 families. However, our understanding of *PLS3*'s specific role in bone metabolism and how it exerts its effects is still poor. To study this further we investigated 3 children from 2 different families with *PLS3* deletions.

Material and Methods: Family 1 consisted of 2 brothers, 11 and 7 years old, and their healthy parents. For the older brother, a transiliac bone biopsy was taken and extensively analyzed using histomorphometry, Quantitate Backscattered Electron Imaging and Raman microspectroscopy. Family 2 consisted of a 12-year-old boy, his osteoporotic mother and healthy father. Massive parallel sequencing and array-CGH was used to genetically evaluate all subjects.

Results: In both families the affected boys had childhood-onset osteoporosis with severe spinal involvement and multiple compression fractures. Both brothers in Family 1 had a deletion of exon 4-16 in *PLS3*, which was inherited from their mother. The index boy in Family 2 had a larger deletion, also inherited from his mother, which spanned the entire *PLS3* gene. All children had a normal biochemical profile. Results from the bone tissue analyses showed a strong hypomineralization of the bone and a striking increase in osteoid volume, osteoid thickness and mineralizing lag time.

Conclusion: Our results indicate that *PLS3* deletions lead to severe spinal osteoporosis and defective bone matrix mineralization, and suggest that *PLS3* is directly involved in the mineralization process.

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P04.74B

Post axial polydactyly: isolated symptom or part of a syndrome. A case of BBS8 with two novel molecular anomalies

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Background. Polydactyly can be isolated or encountered in more than 300 syndromes, mostly ciliopathies.

Methods. A young girl aged 5 was investigated for dysmorphic features, speech delay, obesity, strabismus and post axial polydactyly of the four limbs.

Results. Array CGH revealed a *de novo* heterozygous duplication of uncertain significance in the 13q31.3 region including the gene *GPC6*. A panel analysis for Bardet Biedl Syndrome (BBS) revealed a new maternally inherited pathogenic splice site mutation in intron 13 of the *TTC8* gene (c.1317+1G>A) and a likely pathogenic splice site variant in the exon 5 of the *TTC8* gene inherited from the father (c.459G>A (p.Thr153Thr)).

Discussion. A previous publication of a case of PAP-A2 with a similar duplication has postulated *GPC6* as a candidate gene for PAPA-A2. For our patient, the additional symptoms have led to test for BBS. This analysis showed a never described mutation: a new pathogenic splice site mutation in intron 13. According to the algorithms, the variant c.1317+1G>A leads to a loss of the natural splice site leading to exon skipping, inclusion of intronic sequences or usage of a cryptic splice site.

Conclusion. We describe a girl with a BBS8 with two novel molecular anomalies: a new splice site mutation in intron 13 of *TTC8* and a duplication including *GPC6*, a candidate gene in the pathogenesis of PAP-A2. The patient's polydactyly is a feature of BBS, but the duplication of *GPC6* has probably influenced the phenotype of the patient, with polydactyly of all four limbs.

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P04.76D

The inflammasome pathway is involved in PXE through IL1B upregulation in patients with a severe cardiovascular PXE phenotype

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Introduction: Pseudoxanthoma elasticum (PXE), an autosomal recessive ectopic mineralization disorder, causes skin, eye and cardiovascular symptoms. The disease shows

striking phenotypic variability without underlying genotype-phenotype correlations. Therefore, we evaluated the collective modifying effect of rare variants on the PXE cardiovascular disease severity.

Methods: Whole Exome Sequencing and burden tests (SKAT-O and C-alpha) were performed in 12 PXE patients with an extreme cardiovascular phenotype (severe/mild). Functional validation included inflammasome stimulation with LPS/ATP in dermal fibroblasts of PXE patients with extreme cardiovascular phenotypes (4 severe/6 mild) and 2 healthy controls. Readout comprised qPCR analysis for *NLRP1* and inflammasome outcome parameter *IL1B*.

Results: Sixteen (SKAT-O) and 74 (C-alpha) genes were identified as significant modifiers of the PXE cardiovascular disease and were enriched for 3 pathways: calcium homeostasis, vascular disease and apoptosis. One modifier, *NLRP1*, is linked to vascular disease and apoptosis, and was withheld for functional validation. IL1B was upregulated in dermal fibroblasts of PXE patients with a severe versus mild cardiovascular phenotype (fold change (FC) +/- 8; Mann-Whitney (MW) test: $p < 0.001$) and a severe phenotype versus healthy controls (FC +/- 51; MW test: $p < 0.001$). In addition, baseline IL1B expression was significantly higher in PXE patients versus healthy controls (FC +/- 10; MW test: $p < 0.001$).

Conclusion: This study provides functional validation for a predicted modifier gene in the PXE cardiovascular phenotype, and for the first time implicates inflammasome involvement in the PXE pathophysiology. This will help to direct future research on the mechanisms underlying PXE and related soft tissue calcification disorders (FWO14/ASP/084).

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P04.78B

Association analyses of functional *NCF1* variants in psoriatic arthritis and psoriasis vulgaris

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Psoriatic arthritis (PsA) and psoriasis vulgaris (PsV) are common chronic inflammatory disorders of complex etiology. In a mouse-model, psoriasis-like skin and joint symptoms were aggravated in a NADPH oxidase deficient strain, indicating regulation by reactive oxygen species (ROS). A subunit of the human NADPH oxidase is encoded by *NCF1*, a gene located in a structurally complex genomic region on chromosome 7q11.23 including two homologous pseudogene copies. The considerable homology of the genomic region comprising *NCF1*, its pseudogenes and further genes predisposes to genomic rearrangements resulting in variability of copy number (CN). A reduced *NCF1* CN and a functional missense variant (c.269G>A/p.Arg90His) in *NCF1*, causing a reduced ROS production, have recently been identified as strong genetic risk factors in other autoimmune diseases. Those associations combined with the animal model prompted us to analyze both variants in 1,248 PsA, 1,157 PsV patients and 932 controls. *NCF1* and pseudogene CNs were determined by qPCR, the functional variant was genotyped with a nested PCR strategy. We did not observe evidence for association with the *NCF1* CN nor with the functional variant in carriers of the most frequent CN ratio (4 pseudogenes : 2 gene copies) despite 97% power to detect nominally significant association with PsA or PsV with c.269G>A/p.Arg90His. The negative findings make a role of these functional *NCF1* variants in psoriasis unlikely, but since oxidative burst seems to play a role in autoimmune disorders, other pathways resulting in deviant ROS production might play a role in the pathogenesis of the disease.

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P04.79C

Evidence of common and differential genetic biomarkers for Ps and PsA

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Introduction: Collagens provide stability and resilience to extracellular matrix of connective tissues, including dermis, blood vessels and bone. Therefore, collagen genes could be involved in the etiopathogenesis of Psoriasis (Ps, OMIM#177900) and Psoriatic Arthritis (PsA, OMIM#607507), which display dysfunction and instability of the connective tissues. *COL10A1* (rs3812111, A/T), *COL6A5* (rs12488457, A/C), *COL8A1* (rs13081855, G/T) and the *miR-146a* (rs2910164, G/C) were selected as potential biomarkers for Ps and PsA susceptibility.

Materials and Methods: 393 Ps, 424 PsA and 600 controls were genotyped by Real Time-PCR and subjected to biostatistic analysis using chi-square test and evaluation of ORs. The potential pathogenetic impact of the associated genes was then investigated by bioinformatic tools.

Results: rs12488457 (A/C, *COL6A5*), rs13081855 (G/T, *COL8A1*) and rs2910164 (G/C, *miR-146a*) were associated with both Ps [rs12488457: $p = 2.97*10^{-9;}$, OR (C): 1.75, CI95%: 1.44-2.13; rs13081855: $p = 0.001$, OR (T): 1.79, CI95%:1.24-2.59; rs2910164: p=0.01, OR (G): 1.29, CI95%:1.04-1.61] and PsA [rs12488457: $p = 1.24*10^{-5}$, OR (C): 2.46, CI95%: 2.03-2.97; rs13081855: p=9.06*10^{-6}, OR (T): 2.17, CI95%:1.53-3.06; rs2910164: p=0.04, OR (G): 1.23 CI95%:1.0-1.51], while rs3812111 (A/T, *COL10A1*) showed significant association with PsA only [$p = 0.008$, OR (T):1.29, CI95%:1.07-1.57]. The bioinformatic analysis reported that *COL6A5*, *COL8A1* and *miR-146a* may be potentially involved in alteration of the proliferation, neovascularization and inflammation pathways leading to Ps and PsA. On the other hand, *COL10A1* was implicated in bone metabolism mechanisms which are dysregulated in PsA.

Conclusion: *COL6A5*, *COL8A1, miR-146a* and *COL10A1* represent new susceptibility biomarkers for Ps and PsA, reflecting thereby the existence of differential mechanisms underlying the etiopathogenesis of these pathologies.

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Analysis of the genetic background of psoriasis in a Hungarian cohort

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Introduction: Psoriasis is chronic, inflammatory disorder of the skin, often associated with arthritis and systemic co-morbidities. Previous genetic studies established that psoriasis susceptibility has strong genetic components beside environmental risk factors.

Materials and Methods: 19 SNPs in 15 candidate genes were genotyped in 782 psoriasis patients and 2000 controls and their association with psoriasis was assessed in a case-control study. Departures from HWE were tested using Fischer exact test. Allelic and genotypic association tests were performed by using either χ^2 test or Fisher's exact test, then allelic and genotypic odds ratios with 95% confidence intervals were calculated, the model was adjusted to confounding factors, too. Association of inferred haplotypes with psoriasis was assessed by a log-additive model. Pathway and network analysis was also carried out.

Results: 12 SNPs in 11 candidate genes showed significant association with psoriasis susceptibility. Six SNPs have protective effect and six confer increased risk for psoriasis revealing modest effect on phenotype. The same variants were found to be significantly associated with the early onset of psoriasis. Protecting and sensitizing haplotypes were identified on chromosomes 1, 5, 6 and 7.

Conclusion: Network analysis of disease associated genes - LCE3D, PLCL2, IL12B, TNF, TRAF3IP2, TNFAIP3, IL6, PON1, FTO and SPATA2 - identified six functional modules centered around TNF, TNFAIP3, IL12B/IL6, SPATA2, PON1 and PLCL2. These results underlie the importance of TNF and NFκB signaling pathways, cytokine production and inflammatory response in psoriasis patients and hints connections to obesity, asthma, atherosclerosis, and IBD among others.

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P04.81A

Expanding the clinical and mutational spectrum of Roberts Syndrome with previously unreported endocrine findings

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Introduction: Roberts/SC Phocomelia syndrome (MIM 268300,269000) is a rare autosomal recessive disorder characterized by limb anomalies, craniofacial dysmorphism, pre- and postnatal growth failure, developmental delay and a variety of visceral anomalies. Roberts syndrome is part of a spectrum named ''cohesinopathies'' with varying phenotypic expression, and is caused by mutations in *ESCO2*. *ESCO2* encodes a protein that establishes sister chromatid cohesion during S phase. In this study, we report on 4 new patients with previously unreported endocrine findings.

Material and Methods: Three patients clinically and radiologically diagnosed with Roberts syndrome, were screened for *ESCO2* variations using Sanger sequencing analysis while one patient diagnosed with FATCO syndrome underwent whole exome sequencing.

Results: Sanger sequencing revealed 3 pathogenic mutations, including a novel mutation identified in a conserved region of *ESCO2*. While the patient with novel c.1433_1437delCTTTT mutation had severe intellectual disability, the other two patients with previously reported c.1131+1G>A splice donor mutation presented with generalized decreased bone mineral density and hypothyroidism, respectively. Finally in the patient with clinical findings resembling FATCO syndrome, WES analysis revealed c.879_880delAG. In this individual premature adrenarche without significant hormonal imbalance was found and this finding was attributed to the Roberts syndrome.

Conclusion: This study demonstrates that premature adrenarche and hypothyroidism may accompany Roberts syndrome. ESCO2 expression in thyroid and adrenal glands as well as the previous description of thyroid agenesis, hypothyroidism and osteoporosis in another ''cohesinopathy'' Cornelia de Lange syndrome further support this observation. This study provides further evidence that patients with Roberts syndrome might be evaluated endocrinologically during their follow-up.

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P04.82B

Skeletal abnormalities in SATB2-associated syndrome

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The *SATB2*-associated syndrome (SAS) has been recently proposed as a new clinically recognizable syndrome that results from deleterious alterations of the SATB2 gene in humans. The characteristic features are intellectual disability with absent or limited speech development, behavioral problems, craniofacial abnormalities with cleft or high palate, and dental abnormalities. About fifty patients have been reported in the literature. Skeletal abnormalities such as tibial bowing, bone fragility or osteoporosis have been reported in patients, suggesting a higher frequency of skeletal complications in SAS. The role of SATB2 in the regulation of skeletal development has recently been demonstrated. Indeed, SATB2 is a regulator of the Osx promoter, itself responsible for the differentiation of mesenchymal cells into osteoblasts. In this context, we decided to perform a non-interventional, multicentric research to understand mechanisms that lead to osteopenia in SAS patients. For all patients, we carried out a complete phosphocalcic assessment including markers of bone formation and bone resorption. We also reviewed skeletal radiographies and bone densitometry when available. We report here our data of 23 patients with SAS (21 with

intragenic pathogenic variant and 2 with a microdeletion) aged from 4 to 33 years. A clinically significant fracture history was present in 10 patients. Osteopenia was present in skeletal radiographies of 10/10 patients. Low mineral density ascertained by osteodensitometry was present in 4/10 patients. We hope that ongoing results will allow better clinical management of SAS patients.

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P04.83C

Identification of novel candidate genes for idiopathic short stature using whole exome sequencing

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Short stature affects 3% of the population. We recently demonstrated that exome sequencing is able to identify disease causing mutations in known short stature genes in 19% of patients. We now performed exome sequencing in 211 clinically characterized families without mutations in known short stature genes to identify potential candidate genes. Variants were assessed for a potential effect on the gene and its product using multiple lines of evidence including expression in chondrocytes. We found 21 genes mutated in at least two patients. Six were especially strong candidates (CPZ, EDEM3, FBRS, RASA3, SLC7A8 and USP45) with mutations in at least two patients. The resulting proteins participate in protein degradation, transcriptional regulation and protein transport. One outstanding candidate gene, *RASA3*, is a member of the RAS-MAPK pathway. Mutations in other members of this pathway are known to cause diseases of the RASopathy complex, with short stature as a main symptom. The 2 patients carrying *de novo* mutations in *RASA3* share some clinical characteristics with other RASopathy patients. We recently identified a third patient with a *de novo* mutation in *RASA3*. As GH treatment is discussed for some RASopathy patients, successful application in one of our patients suggested that GH might be indicated in the treatment of these patients. In conclusion, using exome analyses in patients with idiopathic short stature, we found 21 strong candidate genes in 40 patients. Thus, Exome sequencing can therefore be of great value to identify the underlying genetic cause in these individuals.

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P04.84D

SJOGREN-LARSSON SYNDROME IN TWO SISTERS WITH AN INTRONIC VARIANT OF ALDH3A2 GENE: CLINICAL FINDINGS

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Introduction: Sjögren-Larsson syndrome is a neurocutaneous disease due to an inborn anomaly of lipid metabolism

and characterized by congenital ichthyosis, intellectual deficit and spasticity. Half of the patients approximately are not able to walk. It is due to mutations of the ALDH3A2 gene (17p11.2), which encodes the enzyme required in the oxidation of fatty alcohols in fatty acids. Transmission is autosomal recessive. They usually live to adulthood requiring care. Neurological symptoms and intellectual deficit do not evolve after puberty. Early symptomatology suggests more serious involvement. GEN: ALDH3A2

Case report: Our index case was a 20-year-old female patient with neurological problems (seizures and spasticity) and congenital bone malformations. She presented a dry, rough, and scaly with a brownish or yellowish tone skin in the trunk and extremities, hyperpigmented hyperkeratotic plaques predominantly in flexures and abundant scalp scaling. She had a 15-year-old sister with the same symptomatology, but who also has a spastic, scissor gait, mental retardation and most severe skin lesions. A genetic study was carried out, detecting the variant c.471 + 1delG in the ALDH3A2 gene in homozygous state. This variant is described as pathological with an autosomal recessive pattern of inheritance, which confirms at the genetic level the clinical suspicion of SJOGREN-LARSSON SYNDROME. The variant detected is also present in sister of the patient with the same clinical diagnosis but with a most severe phenotype. Genetic confirmation is of great importance in these patients, due to the clinical follow-up they require, and offers the possibility of assessing genetic counseling.

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P04.85A

SHOX haploinsufficiency presenting with isolated short long bones in the second and third trimester

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Haploinsufficiency of the transcription factor Short Stature Homeobox (*SHOX*) manifests as a spectrum of clinical phenotypes, ranging from disproportionate short stature and Madelung deformity to isolated short stature. Here, we describe five infants with molecularly confirmed diagnoses of *SHOX* haploinsufficiency who presented *in-utero* with short long bones during routine antenatal scanning from as early as 19 weeks gestation. Other fetal growth parameters were normal. The molecular basis of *SHOX* haploinsufficiency was distinct in each case. In four cases *SHOX* haploinsufficiency was inherited from a previously undiagnosed parent. In our *de novo* case, *SHOX* haploinsufficiency reflected the formation of a derivative sex chromosome during paternal meiosis. Final adult height in the *SHOX* deficient parents ranged from -1.9 to -1.2 SDS. All affected parents had disproportionately short limbs and two affected mothers had bilateral Madelung deformity. To our knowledge, *SHOX* haploinsufficiency has not previously been reported to present *in-utero*. Our experience illustrates that *SHOX* deficiency should form part of the differential diagnosis of fetal short long bones and suggests a low threshold for genetic testing. This should be particularly targeted at, but not limited to, families with a history of features suggestive of *SHOX* deficiency. Data on the postnatal growth of our index cases is presented which demonstrates that antenatal presentation of *SHOX* haploinsufficiency is not indicative of severe postnatal growth restriction. Early identification of *SHOX* deficiency will enable accurate genetic counselling reflecting a good postnatal outcome and facilitate optimal initiation of growth hormone therapy.

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P04.86B

Functional missense and splicing variants in the retinoic acid catabolizing enzyme CYP26C1 in idiopathic short stature

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Height is a complex quantitative trait with a high heritability. Short stature is diagnosed when height is significantly below the average of the general population for that person´s age and sex. We have recently found that the retinoic acid degrading enzyme CYP26C1 is a modifier for SHOX deficiency phenotypes towards more severe clinical manifestations. Here, we asked whether damaging variants in *CYP26C1* alone could lead to short stature. We performed exome and Sanger sequencing to analyze 856 individuals with short stature where SHOX deficiency was previously excluded. Three different damaging missense variants and one splicing variant were identified in six independent individuals; the functional significance of the identified variants was tested *in vitro* or *in vivo* using Zebrafish as a model. The genetic and functional data reported here indicate that *CYP26C1* represents a novel gene underlying growth disorders and that damaging variants in the absence of *SHOX* mutations can lead to short stature.

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P04.87C

Clinical, demographic and nosologic characterisation of the genetic disorders of the skeleton in Turkey: The skeletal dysplasia registry

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Introduction: We aimed to determine the clinical, demographic and nosologic characteristics of the genetic disorders of the skeleton in Turkey over the past 13 years (February 2005-February 2018).

Materials and Methods: "The Skeletal Dysplasia Registry" was established in 2005 at Hacettepe University Pediatric Genetics Department, a tertiary reference center for all pediatric subspecialties. The registry mainly included patients with the genetics disorders of the skeleton. Age at diagnosis, geographical region of referral, family history, parental consanguinity, clinical diagnosis, molecular diagnosis and inheritance patterns when available were reviewed respectively. The study was approved by Hacettepe University Noninterventional Clinical Research Ethics Board (Ref No: GO 17/321).

Results: The findings of a total of 884 patients were reviewed. The overall consanguinity rate in our study is 52% with some regional differences. A comparison is made in two time periods, 2005-2009 and 2009-2018. The registry has enabled scientific contributions through clinical, radiographic and molecular delineation of different clinical diagnosis including identification of founder mutations in *FKBP10* in autosomal recessive OI and *SFRP4* mutations in Pyle disease. Additional patients with recently identified genes including *RSPRY1*, *EXTL3*, *XYLT2*, *EXOC6* and *PGAP3* were also diagnosed and registered during this time period.

Conclusions: The present study provides further information about the clinical and nosological characteristics of the genetic disorders of the skeleton in Turkey. Despite limitations this registry still represents the first of its own kind from Turkey and around, and is a useful tool not only for the physicians but also for the families.

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P04.88D

Duplication of 10q24 locus: broadening the clinical and radiological spectrum

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Split hand-split foot malformation (SHFM) is a rare condition that occurs in 1 in 8500-25000 newborns and accounts for 15% of all limb reduction defects. SHFM is heterogeneous and can be isolated, associated with other malformations or syndromic. The mode of inheritance is mostly autosomal dominant with incomplete penetrance, but can be X-linked or autosomal recessive. Seven loci are currently known: SHFM1 at 7q21.2q22.1 (*DLX5* gene), SHFM2 at Xq26, SHFM3 at 10q24q25, SHFM4 at 3q27 (*TP63* gene), SHFM5 at 2q31 and SHFM6 as a result of mutations in *WNT10B* (chromosome 12q13). Duplications at 17p13.3 are seen in SHFM when isolated or associated with long bone deficiency. Tandem genomic duplications at chromosome 10q24 involving at least the *DACTYLIN* gene are associated with SHFM3. No point mutation in any of the genes residing within the region has been identified so far, but duplication of exon 1 of the *BTRC* gene may explain the phenotype, with likely complex alterations of gene regulation mechanisms that would impair limb morphogenesis. We report on 32 new index cases identified by array-CGH and/or by qPCR, including some prenatal ones, leading to termination for the most severe. Twenty-three cases were presenting with SHFM and 7 with monodactyly only. Two had an overlapping phenotype. Additional findings were identified in 5 (renal dysplasia, cutis aplasia, hypogonadism and agenesis of corpus callosum with hydrocephalus). We present their clinical and radiological findings and review the literature on this rearrangement that seems to be one of the most frequent cause of SHFM.

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P04.89A

A Syrian patient with Steel syndrome due to compound heterozygous *COL27A1* mutations with hitherto undescribed colobomas extending the clinical spectrum

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Introduction: The combination of short stature, bilateral congenital dislocation of the hip, carpal coalition, dislocation of the radial head, cavus deformity, scoliosis, and vertebral anomalies was first described in 1993 by Steel et al. (OMIM #615155) in twenty-three children from Puerto Rico. It is caused by deficient matrix protein collagen 27 alpha 1 expressed in cartilage, skin, and tendons and is inherited as an autosomal recessive trait. Causative mutations in the gene *COL27A1* have been identified primarily as a possible founder effect in Puerto Ricans and in only two more families, in one of which the patient also presented hearing loss.

Here we report a girl aged 9 years born to non-consanguineous Syrian parents with characteristic features of Steel syndrome (short stature, massive malalignment of large joints, kyphoskoliosis, hearing loss) and matching facial dysmorphism (large, laterally extended palpebral fissures, arched eyebrows, flat midface, long philtrum, and short nose with low hanging columella) who also showed bilateral colobomas of the irides, retinae, and uveae with unilateral affection of the macula, which have not been described before. Her intelligence seemed normal, as was an MRI of the brain.

Results: Exome sequencing identified two novel compound heterozygous variants in *COL27A1*: c.93del, p.(Phe32Leufs*71) in exon 2 and c.3075del, p.(Lys1026Argfs*33) in exon 26 in this child. Her parents were confirmed heterozygous carriers.

Conclusions: Our findings extend the clinical spectrum of this exceptionally rare disorder and provide evidence of developmental defects of the eye caused by *COL27A1* mutations.

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P04.90B

Distinct phenotypes within *TRPV4*-associated disorders in the infantile period

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Introduction: TRPV4 is a calcium permeable non-selective cation channel expresses in different tissues. *TRPV4* mutations have been implicated in autosomal dominant diseases of skeletal and peripheral nervous system. Here, we report *TRPV4* mutations in three patients with spondylometaphyseal dysplasia Kozlowski type (SMDK), metatropic dysplasia (MD) and scapuloperoneal spinal muscular atrophy (SPSMA).

Metod and Result: Patient-1 was a 3-year-old girl. She had normal height, short trunk, lumbar scoliosis, generalized severe platyspondyly, left proximal femoral metaphysis irregularity, short femoral necks and delayed carpal ossification which were compatible with SMDK. Progressive scoliosis, waiddling gait and metaphyseal irregularity of right distal ulna and radial bones were developed with aging. At 8.5 years of age her height was -2.5 SDS. Patient-2, a 2-year-old boy had torticollis (noticed in 3rd month), narrow chest with normal stature (-1.7SD), was diagnosed as MD especially shortening of long tubular bones, tail-like sacral appendage and radiological features including defective ossification of servical bodies, platyspondyly, metaphyseal flaring, and delayed epiphyseal ossification. His height is -1.7 SD at 4.5 years of age. Heterozygous *TRPV4* mutations c.1781G>A and c.2396C>G were detected by Sanger sequencing in Patient 1 and 2, respectively. Patient-3, a 1-year-old boy had laryngomalacia, torticollis, hip dysplasia, muscle weakness, bilateral pes equinovarus, and scoliosis. Mild platyspondyly and acetabular irregularity were present radiologically. Using exome sequencing, we identified heterozygous novel mutation in *TRPV4* (c.806G>A). The patient was diagnosed SPSMA phenotype.

Conclusion: This study showed both neuromuscular diseases and skeletal dysplasia due to TRPV4 mutation in infantile period should be kept in mind.

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P04.91C

Transcriptome analysis of skin fibroblasts with dominant negative *COL3A1* mutations provides insights into the molecular pathology of vascular Ehlers-Danlos syndrome

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Vascular Ehlers-Danlos syndrome (vEDS) is a dominant inherited connective tissue disorder caused by mutations in the *COL3A1* gene encoding type III collagen (COLLIII), the major expressed collagen in blood vessels and hollow organs. The majority of *COL3A1* causative variants are glycine substitutions and in-frame splice mutations in the triple helix domain that through a dominant negative effect are associated with the severe clinical spectrum characterized by fragility of soft connective tissues with arterial and organ ruptures. Herein, we performed gene expression profiling in cultured skin fibroblasts from three patients with different structural *COL3A1* mutations. Transcriptome analysis revealed significant expression changes of several genes involved in maintenance of endoplasmic reticulum (ER) homeostasis, COLLs folding, extracellular matrix (ECM) organization, proteasome complex, and cell cycle regulation. Protein analyses showed that aberrant COLLIII expression causes the disassembly of many structural ECM constituents, such as fibrillins, EMILINs, elastin, perlecan, decorin, and versican, all playing a crucial role in the vascular system. Furthermore, the altered distribution of the ER marker protein disulfide isomerase PDI and the strong reduction of the COLLs-modifying enzyme FKBP22 are consistent with the disturbance of ER-related homeostasis and COLLs post-translational modifications, indicated by microarray. Our findings provide a picture of the gene expression changes in vEDS skin fibroblasts and highlight that dominant negative mutations in *COL3A1* affect maturation and deposition into the ECM of several structural proteins crucial to the integrity of soft connective tissues, and that ER dysfunction might play an important role in the etiology of this severe vascular disorder.

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P05 Cardiovascular disorders

P05.01A
Genetic variants in familial abdominal aortic aneurysms

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Introduction: Abdominal Aortic Aneurysm (AAA) has a prevalence of 5% in the elderly population. An AAA occurs when the aorta below the renal arteries expands to a diameter of 3cm or more. In the Netherlands, each year approximately 5000 AAA patients are hospitalized and around 750 people die due to AAA rupture.

Hypothesis: Approximately 20% of AAA patients are familial and our hypothesis is that genetic predisposition is a significant cause for abdominal aneurysm pathology. Our goal is to identify the genes that play a role in the formation of AAA.

Methods: Our study population consists of approximately 1250 AAA patients. So far we sequenced 548 of these AAA patients. Complete Genomics whole genome sequencing (WGS) was performed in 3 families (15 individuals)and whole exome sequencing (WES) on the illumina platform using Agilent Haloplex and CRE sureselect exome capturing technology was performed in 71 families (175 individuals) and 358 single familial AAA patients. Burden analysis was used to identify genes enriched in our AAA population.

Results: We present the detailed workflow of the analysis of the genomics data. We will discuss several candidate genes identified such as the enrichment for variants we identified in the COL4A2 gene.

Conclusions: In 118 out of 512 families a variant in a diagnostic AAA gene was found. Analysis of all genes in the exome dataset led to the identification of several candidate genes that show variants in more than one AAA family and that have not been linked to AAA before.

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P05.02B
Targeted massively parallel sequencing of a representative cohort of Czech patients with various rare aortopathies demonstrates the clinical utility of genetic testing and the need for a multidisciplinary approach to at risk families

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Introduction: Aortopathies represent heterogeneous group of rare inherited disorders with a variable phenotype ranging form of aortic aneurysm/dissection with/without associated cardiac valvular disease. We studied the distribution of variants within selected candidate genes in a representative cohort of Czech paediatric-/adult patients.

Materials and Methods: Massively parallel sequencing was performed in 120 unrelated individuals (average age 42,5 years) using a custom-made panel comprising either 136 or 229 cardiac/aortic conditions-related genes (NimbleGen/Illumina). Detected variants were validated by Sanger DNA sequencing and segregation analysis. In 40 "sequencing-negative" cases CNVs in the *FBN1, TGFBR1* and *TGFBR2* genes were examined by MLPA (MRC-Holland).

Results: Pathogenic/likely pathogenic DNA variant (Class ≥ 4) were found in 10/120 (8.3%) cases, while VUS (Class 3) were detected in 40/120 (33.3%) patients comprising genes *FBN1, NOTCH1, FBN2, MYH11* and others. Majority of pathogenic variants were observed in *FBN1* (20.0%), while CNVs were not identified. Interestingly, pathogenic variants were also observed in 7/120 (5,8%) patients within aortopathy-unrelated genes conferring other cardiovascular risks.

Conclusions: As expected majority of variants were identified in connective tissue-related genes. The overall lower variant detection rate corresponds to published data. The detection of pathogenic or potentially pathogenic variants for other cardiac conditions (e.g. arrhythmias) demonstrates the diagnostic usefulness of broader gene panels. Segregation analysis together with clinical examination of positive cases increases the utility of DNA sequencing, thereby underscores the multidisciplinary character of our approach and usefulness of cooperating with compliant at risk families. Supported by 00064203, CZ.2.16/3.1.00/24022, LD14073, IGA NT13770.15-27682A and IKEM: 00023001; 9039.

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P05.03C
MicroRNAs in Arrhythmogenic Cardiomyopathy: from tissue-profile to circulating-signature

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Background: Arrhythmogenic cardiomyopathy (AC) is a clinically and genetically heterogeneous disease, characterized by progressive myocardial fibro-fatty replacement and high risk of sudden cardiac death. Half of AC patients harbor private desmosomal gene mutations. MicroRNAs (miRNA) have been associated with numerous pathophysiological conditions, as gene-expression regulatory molecules. Their role in AC is largely unknown.

Methods: The study cohort comprised 59 genotype-positive AC-subjects and 24 healthy controls (Ctrl). 84-miRNA array analysis was carried out on frozen right-ventricle myocardial tissue samples derived from 8 AC heart-transplanted patients; 9 AC-whole blood samples, and 6 Ctrls. miRNA validation was performed by qPCR (ΔΔCt method) on 42-AC and 18-Ctrl.

Results: miRNA profiling on AC-tissue samples displayed a genotype-related profile showing 19 differentially expressed miRNAs in PKP2 carriers, 15 in DSP carriers and 14 in DSG2 carriers. A common signature was identified between PKP2 and DSP carriers (PKP2/DSP profile), different from DSG2 profile. *In silico* target prediction of both profiles marked Hippo Signaling Pathway (p-value 1.6e-9 and 6.4e-6). Analysis of AC-tissue sample-data as a unique group confirmed 26 miRNAs (AC-tissue profile) with predicted targets in the AC pathway (p-value 0.01). AC-blood miRNA profiling showed a 14-miRNA signature, of which 10 miRNAs were found also in AC-tissue profile.

Conclusions: A genotype-related miRNA profile was observed on AC-tissue samples, as to reflect clinical variability. In addition, 10 miRNAs in common were identified between AC-tissue and AC-blood profiles, demonstrating a specific AC-miRNA signature. *In silico* analysis highlighted pathways involved in AC pathogenesis demonstrating a key role of miRNAs in AC.

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P05.04D
Differential gene expression analysis in Arrhythmogenic Cardiomyopathy

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Background. Arrhythmogenic cardiomyopathy (AC) is an inherited myocardial disease characterized by fibro-fatty replacement of the myocardium and life-threatening arrhythmias caused mainly by low penetrant mutations in desmosome-encoding genes. The molecular mechanism underlying disease pathogenesis is still unclear. To determine molecular pathways underlying disease onset, gene expression profiling was performed on AC patients and transgenic mice with a desmoglein-2 (dsg2) mutation.

Methods. RNA-Seq was carried out, separately, on the right (RV) and the left (LV) ventricle of 9 AC heart-transplanted patients carrying pathogenic desmosomal mutations paired with 6 age-matched nondiseased donors. RNA-Seq was carried out also on 8 transgenic mice over-expressing NS-dsg2 mutation (TgNS) at 2 different age-groups (<2weeks and >3weeks), to deduct genetic/epigenetic interference-factors and on a paired age-matched control group comprised 6 over-expressing wild type dsg2 (TgWt) and 2Wt mice.

Results. 1136 and 822 differentially expressed genes (DEGs) were respectively identified in the RV and LV of AC patients. Further, 143 DEGs were identified comparing TgNS<2weeks and TgNS>3weeks gene expression profiling. Finally, 82 DEGs were shared comparing human and murine (TgNS>3weeks) expression-profiling among which genes most linked to the suppression of the canonical WNT/β-catenin and the activation of TGF-β signaling pathways. However, 29 DEGs were identified in TgNS<2weeks comparing them to age-matched controls (WT<2weeks and TgWt < 2weeks).

Conclusions. Transcriptome profiling enabled the identification of the culprit molecule aiding cell-cell contact detachment under stress conditions and wound healing repair through suppression of the WNT/β-catenin signalling pathway.

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P05.06B
The effect of the expression level of tissue inhibitor of metalloproteinase-3 on the development of atherosclerosis in patients with myocardial infarction

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TIMP3, a member of the Tissue Inhibitors of Metalloproteinases (TIMPs) binds to components of the extracellular matrix and forms insoluble complex. TIMP3 has a crucial regulatory role in adipose tissue. Our aim is to reveal the relationship between *TIMP3* expression and atherosclerosis pathogenicity in peri-coronary epicardial adipose tissue (EAT) and circulating leukocytes of patients with myocardial infarction (MI). Methodologically, the expression levels of *TIMP3* were investigated in patients with MI ($n = 46$) who had atherosclerosis severity determined by SYNTAX and GENSINI scores that compared with the control group ($n = 24$). The expression levels of *TIMP3* in the leukocytes ($n = 69$) and peri-coronary EAT ($n = 23$) obtained from coronary artery bypass graft surgery using qRT-PCR. The expression results were analyzed using the comparative CT method, and results were statistically evaluated. Previously unpublished findings showed that *TIMP3* expression levels were significantly lower in post-mortem advanced atherosclerotic plaques than in normal arteries ($p < 0.05$) and that TIMP-3 protein was present in plaque enriched with macrophages of the coronary artery sections. In this study, it was determined that expressions of *TIMP3* increased 1.25 fold in peri-coronary EAT and decreased 4.5 fold in circulating leukocytes as compared to control samples ($p > 0.05$). The data were evaluated in detail according to conventional risk factors. In conclusion, *TIMP3* expression levels in leukocytes and fat tissue affecting the development of atherosclerosis were evaluated in patients with MI. These analyses are still ongoing in more patient samples. This study was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project numbers:28473 and 21496)

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P05.07C

Identification of novel loci for heart rate response to exercise and recovery

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Introduction: Reduced heart rate (HR) responses to exercise (ΔHRex) and to recovery (ΔHRrec) are associated with higher cardiovascular mortality rates, possibly due to abnormalities in autonomic balance. We aimed to discover single-nucleotide polymorphisms associated with both indices and to identify associated biological pathways.

Methods: A total of 67,257 participants in an exercise test from the UK Biobank study were included. We calculated differences in HR at peak exercise (ΔHRex) and at 1-minute post-peak exercise (ΔHRrec) with respect to resting HR. Next, we randomly divided participants into discovery ($N = 40{,}000$) and replication ($N = 27{,}257$) cohorts and we performed a genome-wide association study (GWAS) for each trait in the discovery dataset and validated the findings in the replication cohort. We finally conducted a combined meta-analysis of GWAS using the full cohort for both traits.

Results: We robustly validated six and eight independent SNPs for ΔHRex and ΔHRrec, respectively. The combined analysis revealed a further eight and seven SNPs for each respective trait that were genome-wide significant ($P < 5x10^{-8}$). In total, 14 and 15 SNPs were identified for ΔHRex and ΔHRrec, respectively, with eight SNPs being common across traits. Bioinformatics analyses highlighted neural development and adrenergic modulation by the autonomic nervous system pathways.

Conclusion: Our results demonstrate that ΔHRex and ΔHRrec are genetically modulated. Our biological findings support the potential link between genetics and autonomic modulation and highlight several plausible candidate genes for both traits. Future studies will confirm the contributions of our identified genetic variants to cardiovascular risk.

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P05.08D

Gene expression signature in human immortalized venous endothelial cells, human coronary artery and human internal thoracic artery endothelial cells exposed to different types of mineral-organic nanoparticles

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Introduction: Mineral-organic nanoparticles (bions) are a result of increasing concentration of precipitating ions or failure in the mechanism of their excretion from human body. It was found that the atherosclerosis and heart valves calcification risk factors are similar to ones of bions formation. This indicates that bions play role in the pathogenesis of cardiovascular calcification.

Materials and Methods: Confluent cultures of human immortalized venous endothelial cells (EA.hy 926), human coronary artery (HCAEC) and human internal thoracic artery endothelial cells (HITAEC) exposed to different types of bions (magnesium phosphate bions [MFB], spherical calcium phosphate bions [SCFB], and needle-like calcium phosphate bions [ICPB]) were used in this study. Expression of LDLR, VLDLR, SCARF1, NOS3, PXDN genes was evaluated by RT-qPCR. Results were normalized by three housekeeping genes (ACTB, GAPDH, B2M). Expression level was calculated by Pfaffl method.

Results: 2-fold decreasing of SCARF1 expression was detected in EA.hy 926 culture exposed to SCFB. In HCAEC culture we found no significant differences in gene expression signature. In HITAEC culture the exposure by all types of bions caused an increase in VLDLR gene expression. HITAEC cultures exposed by all types of mineral-organic nanoparticles are characterized by enhanced expression of all studied genes compared to HCAEC.

Conclusions: Exposure by mineral-organic nanoparticles can lead to changing in gene expression signature in different types of endothelial cells depending on type of bions. HITAEC are more sensitive to such exposure. The reported study was funded by Russian Foundation for Basic Research according to the research project № 17-04-00570.

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P05.09A

Assessment of CNVs in dilated cardiomyopathies by whole exome sequencing

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Genetic testing for inherited cardiomyopathies has improved in the last decade using next generation sequencing (NGS). Although CNV of large segments of DNA may significantly affect transcription and translation of cardiomyopathic genes, these abnormalities remain often unrecognized even with the use of NGS.

We have performed whole exome sequencing in a cohort of 323 patients with dilated cardiomyopathy. We used a read-depth coverage strategy to call CNVs from the short-read sequence data. Detected CNVs were then validated by q-PCR, RT-PCR and western blot analysis. The prevalence of major CNVs in the cohort was 2%. We have found large deletions in *DMD*, *LAMP2*, *FLNC* and *MYH7* genes, which were predicted to cause major structural and functional abnormalities of the affected genes. The corresponding pedigrees and clinical phenotypes will be presented. Assessment of CNVs with whole exome sequencing elucidates genetic architecture in a substantial proportion of patients with dilated cardiomyopathy. It should be a routine part of NGS bioinformatics. Supported by the research grant: AZV-MZ 15-27682A and IKEM 00023001.

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P05.10B

Rare variants in cardiomyopathy related genes - a Portuguese cohort population

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Introduction: With the latter advances in high throughput sequencing technologies there was an increasing demand for broader comprehensive Next-Generation Sequencing (NGS) gene panels to screen for mutations in genetic diseases with heterogeneous etiology. In cardiology, genetic testing has been routinely offered to patients to improve prognosis through appropriate lifestyle and medical interventions. There are now 340 gene entries retrieved under "cardiomyopathy" search in OMIM and 375 genes under the HPO superclass "Abnormal myocardium morphology" (HP:0001637).

Methods: The study population comprised 150 unrelated patients diagnosed with cardiomyopathies, namely Hypertrophic Cardiomyopathy (HCM), Dilated Cardiomyopathy (DCM) and Arrhythmogenic Right Ventricular

Cardiomyopathy (ARVC). Up to 57 genes associated with cardiomyopathies were sequenced, mostly by NGS.

Results: Pathogenic variants were detected in 39 patients and in further 50 patients it was found an uncertain significance/likely pathogenic variant, namely novel variants in 17 patients (11.3%). One novel variant was detected in two unrelated DCM patients in the *LMNA* gene - c.490G>A, p.(Asp164Asn), suggesting an increased frequency in the Portuguese population.

Conclusions: Although genetic analysis through comprehensive multigene NGS approach potentially increases diagnostic rate, it also raises new challenges. Rare benign specific population polymorphisms add extra difficulty regarding pathogenicity classification of variants. Further characterization studies of specific populations could improve classification variants algorithm, preferably within public databases with the possibility to identify variants by nationality.

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P05.11C
Identification of gene mutations in pediatric cardiomyopathy by whole exome sequencing

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Introduction: Primary cardiomyopathy in children is rare but serious condition with a high mortality rate. Hypertrophic and dilated cardiomyopathies are the most common presentations. Etiology has been mainly idiopathic; however, with the use of next generation sequencing techniques, it has been noted that up to nearly half of idiopathic pediatric cases arose from a specific genetic mutation. Therefore, this study aimed to identify genetic causes of primary unexplained cardiomyopathy.

Materials and Methods: Children with primary unexplained cardiomyopathy, ranging from newborns to 20-year olds, were recruited during March 2016 to May 2017 at Thammasat University Hospital. Complete patient history and physical examination data were collected by a geneticist; cardiac examination and echocardiogram was performed by pediatric cardiologists. Whole exome sequencing was performed in all cases.

Results: Fourteen patients were enrolled in our study: 8 cases were dilated type, and 6 were hypertrophic. Two were excluded given identification causes during period of follow-up (hypocalcemia and pace-maker induced dilated cardiomyopathy). 118 gene lists for cardiomyopathy were analyzed in 12 included cases. In cases of syndromic cardiomyopathy, specific genes were added to aid the analysis, but none was detected. Pathogenic and likely pathogenic mutations were identified in 5 patients: *SOS1*, *HRAS*, *TTN, FLNC* and *TXNRD2*.

Conclusion: Our cohort demonstrated that 41% of our cases were not actually idiopathic. In light of this revelation and despite its high cost, genetic testing is also useful in determining genetic risk in the family as well as helping to predict the prognosis of the cardiomyopathy.

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P05.13A
Targeted Next-Generation Sequencing in Patients with Non-syndromic and syndromic Congenital Heart Disease

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Congenital heart diseases are genetically heterogeneous. Targeted next-generation sequencing (NGS) can identify the genetic causes in a significant proportion of the population.

Aim: We tested a targeted NGS specific gene panel in patients, adults and children, with syndromic and non-syndromic cardiac involvement.

Results: The patient cohort included 77 patients(52 males;28.4 ± 22.6years), 41 people with hypertrophic/dilated cardiomyopathy, 3 with arrhythmogenic cardiomyopathy, and 33 with syndromic cardiac involvement (including RASopathies and fibrillinopathies). Amplicon libraries for 174 related genes were generated using the TruSight-Cardio®kit(Illumina,CA) and sequenced using the Illumina MiSeq platform. Sequence data were processed using ANNOVAR software. Thirty-six variants (30 missense, 4

frameshift, 2 splice-site variants) were considered pathogenic or likely pathogenic and 10 were variants of unknown significance (VUS) in MYH7;MYBPC3;MYL2; MYO6; MYPN;ACTC1;BAG3;CSRP3;KCNH2;DSC2;CACNA1C;FBN1;PTPN1;SOS1;BRAF;LPL;ABCC9 and APOE genes. Twenty-five variants present in public databases, with very rare allele frequency, have been previously linked to cardiomyopathy or relevant syndromic phenotypes. Twenty-four were novel mutations, currently not found in public databases, of which 9 were classified as VUS. Two patients carried pathogenic variants for two diseases (heterozygotes in different genes), with possibly synergistic deleterious effects.

Conclusion: First-line targeted genetic NGS testing identified a significant variant (pathogenic or likely pathogenic) for the phenotype, in 51,5%(17/33) of the cases with syndromic cardiac involvement, and in 40.9% (18/44) of the cases with non-syndromic cardiomyopathies, providing the opportunity for diagnostic, risk stratification and prevention, along with genetic counselling.

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P05.14B

The association of genes of fibrogenesis to the development of cardiovascular continuum comorbidity

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The aim of this study was to assess the genetic structure of cardiovascular continuum comorbidity. The study included 531 patients with myocardial infarction (MI) and 285 Russian inhabitants of Siberia. Patients were divided into groups: 113 MI patients without risk factors (arterial hypertension (HT), dyslipidemia (DLE), type 2 diabetes mellitus (T2D)); 146 MI patients with HT; 96 MI patients with HT and HDL; 96 MI patients with HT, HDL and T2D designated as "syntropy of cardiovascular continuum". Genotyping of 58 SNPs was performed on Sequenom MassARRAY (USA). These SNPs are localized in the genes involved in fibrogenesis and/or associated with cardiovascular diseases and atherosclerotic plaque stability. Statistical data analysis was performed in the software environment R. Isolate MI showed the association with genes of various biological pathways: immune response *CD79A* (rs3810153), *IFNGR1* (rs17181457), endothelial dysfunction *KIAA1462*(rs3739998), reparations *LIG1* (rs20579), fibrogenesis (*ADAMDEC1*(rs3765124). In groups with risk factors, it was found that: MI and HT associations with genes involved in fibrogenesis *ITGA4* (rs1143674), *ITGB5*(rs6778643, rs1007856), *ADAMDEC1* (rs3765124), *CDKN2BAS1*(rs1333049) and immune response *IFNGR1* (rs17181457); MI, HT and DLE associations with genes involved in fibrogenesis (*ITGA4* (rs1143674), *ITGB5*(rs6778643), *ADAMDEC1*(rs3765124), *CDKN2BAS1*(rs1333049)), immune response *IFNGR1* (rs17181457), homeostasis of glucose and low-density lipoproteins (*TAS 2R38*(rs1726866), *LDLR*(rs2738446)). "Syntropy of cardiovascular continuum" associations with genes involved in fibrogenesis (*CDKN2BAS1*(rs1333049), (*MTAP*(rs7023329)), endothelial dysfunction *KIAA1462* (rs3739998), lipid metabolism *APOA2*(rs5082). In conclusion, isolate MI and MI with risk factors had different genetic susceptibility profiles. Cardiovascular continuum comorbidity was characterized by genes involved in various biological processes. The study was conducted with the support of the RFBR (№16-04-00840\16).

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P05.15C

KRIT1 loss of function induces a chronic Nrf2-mediated adaptive homeostasis that sensitizes cells to oxidative stress: implication for Cerebral Cavernous Malformation disease

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KRIT1 (CCM1) is a disease gene responsible for Cerebral Cavernous Malformations (CCM), a major cerebrovascular disease of proven genetic origin affecting 0.3-0.5% of the population. Previously, we demonstrated that KRIT1 loss-of-function is associated with altered redox homeostasis, suggesting a novel pathogenic mechanism for CCM disease and raising the possibility that KRIT1 loss exerts pleiotropic effects on multiple redox-sensitive mechanisms. To address this possibility, we investigated major redox-sensitive pathways and enzymatic systems that play critical roles in

fundamental cytoprotective mechanisms of adaptive responses to oxidative stress, including the master Nrf2 antioxidant defense pathway and its downstream target Glyoxalase 1 (Glo1), a pivotal stress-responsive defense enzyme involved in cellular protection against glycative and oxidative stress through the metabolism of methylglyoxal (MG). Experimental outcomes showed that KRIT1 loss-of-function induces a redox-sensitive sustained upregulation of Nrf2 and Glo1, and a drop in intracellular levels of major apoptosis-protective proteins, including MG-modified heat shock protein 70 (Hsp70) and 27 (Hsp27), leading to a chronic adaptive redox homeostasis that counteracts intrinsic oxidative stress but increases susceptibility to oxidative DNA damage and apoptosis. While supporting and extending the pleiotropic functions of KRIT1, these findings shed new light on the mechanistic relationship between KRIT1 loss-of-function and enhanced cell sensitivity to oxidative stress, thus providing valuable new insights into CCM pathogenesis and novel options for the development of preventive and therapeutic strategies.

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P05.16D

Quantification of DNA copy number variations in patients with coronary artery disease by digital droplet PCR

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Introduction: Recently we performed the genome-wide analysis of CNVs in patients with coronary artery disease (CAD) by using array-CGH. We found 90 CNVs, and 13% of them were novel. The aim of this study was to determine the frequencies of the several candidate CNVs (*SFMBT1*, *PRKRA*, and *SIRPB1*).

Materials and Methods: We have extracted DNA specimens from white blood cells of 100 patients with CAD, 100 patients with both CAD and diabetes mellitus 2 type (DM2), and 130 persons without any clinical and laboratory features of atherosclerosis, at the same ages. Digital droplet PCR (QX200) along with TaqMan Assays was used to identify CNVs.

Results: We detected the loss in the 3p21.1 region (*SFMBT1*) in 11.48% patients and 8.5% in control group. However, patients with CAD and DM2 had more frequently loss of *SFMBT1* (16%) than patients with CAD (8%). The frequency of gain 2q31.2 (*PRKRA*) was 6% in patients with CAD whereas in control group we identified it in one person only. The frequency of loss 20p13 (*SIRPB1*) was 67% in both groups.

Conclusion: It is likely that the identified CNV loss in the 3p21.1 region (*SFMBT1*) plays a certain role in the risk of developing CAD and DM2 ($p = 0.006$). At the same time, we detected a trend towards increased frequency of the gain in the 2q31.2 region (*PRKRA*) in patients with CAD.

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P05.18B

Multivariate analysis of comorbidities in congenital heart disease: Making sense of phenotypic heterogeneity

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Introduction: The clinical presentation of congenital heart disease (CHD) is often accompanied by diverse comorbidities within and outside the cardiovascular system. There is significant heterogeneity in the presence of comorbidities and this can make genetic diagnosis and identification of new CHD-associated genes challenging, especially for rare forms of CHD, where cohorts are usually small. This is evident in low diagnostic yields of standard criteria for suspicion of 22q11-deletion syndrome (22q11DS), and limited identification of genes associated with Ebstein Anomaly (EA).

Materials and Methods: Taking advantage of a concentration of cases of rare forms of CHD in a single center in Colombia, unusually large cohorts of patients with suspicion of 22q11DS and EA were assembled. A comorbidity database was constructed and multivariate statistical analysis was carried out to identify correlations between comorbidities in different subgroups in each cohort.

Results: The data show phenotypic heterogeneity in CHD can mask the existence of identifiable subgroups which can be used to improve diagnosis and identify genetic variants in CHD. In patients meeting criteria for 22q11.2DS, multivariate analysis of comorbidities can improve the specificity of clinical evaluations without sacrificing sensitivity. In patients with EA, multivariate analysis revealed a distinct homogenous subgroup with a likely

distinct genetic etiology, presenting with pre-excitation arrhythmias with reduced outflow tract obstruction and improved survival.

Conclusions: Multivariate analysis can reveal clinically relevant patterns in comorbidity datasets of phenotypically heterogeneously disease cohorts. This approach can be used in the identification of novel genetic causes and the improvement of clinical diagnostic criteria.

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P05.19C

Integration of large-scale genomic data sources to identify novel genetic loci for congenital heart disease

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Background: Small nucleotide variants and copy number variants (CNV) have been found to affect congenital heart disease (CHD) risk. Yet, the identification of the genetic causes of CHD remains quite challenging.

Purpose: To integrate data on both classes of variation associated with non-syndromic CHD cases as a better means for identification of candidate genes predisposing to CHD.

Methods: Here, we have updated a previously published CHD case CNV list and generated a control CNV list using: a) DECIPHER database, b) ISCA database, c) ECARUCA database, d) 1000 Genome phase 3 dataset, e) DGV database and f) published literature.

Results: Analysing deleted (del) and duplicated (dup) CNVs independently resulted in unique case CNV regions not present in the controls. Further filtering led to the identification of 54 novel candidate protein-coding genes in del CNVs present only in non-syndromic CHD cases and with high/medium impact variants in exome data from our cohort of Tetralogy of Fallot patients. Moreover, we have identified 50 genes in those unique case CNV regions that were previously shown to be associated with CHD such as *GATA4* for atria septal defects and *NKX2-6* for conotruncal heart malformations.

Conclusions: We demonstrate a promising new strategy with the integration of large-scale genomic data sources to identify novel candidate genes for CHD and their contribution in heart development. We are currently performing functional work with our strongest candidate gene for which there is evidence that knockout mouse has ventricular septal defects and hypoplastic aortic valve.

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P05.20D

Molecular autopsy reveals clues for genetic basis of congenital valve defect

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Introduction: Congenital heart defect (CHD) consists in a large set of functional and structural anomalies that arise during the cardiac embryogenesis including septal defects, valve defects, and outflow tract anomalies. Valve defect is an important cause of mortality and morbidity. However, the genetic basis of congenital valve defect is unclear.

Materials and Methods: We investigate the contribution of genomic alterations in the valve defect`s pathogenesis using molecular methods in 18 cases postmortem of stillbirth and infant from Serviço de Verificação de Óbitos, HC-FMUSP. DNA samples from skin, diaphragm, and heart tissues were evaluated using AmpFℓSTR® MiniFiler™ PCR Amplification Kit (Life Technologies™, California, USA) and Multiplex Ligation-dependent Probe Amplification (MLPA) with different kits (MCR-Holland, Amsterdam, the Netherlands).

Results: In 8 out of 18 stillbirth and infant (44,4%) show alterations in the genome, including trisomy 18 (5 cases), trisomy 21 (2 cases) and duplication of 4p16 (1 case). The tricuspid valve defect was reported in all syndromes describe above. Besides that, the mitral valve defect was associated with deletion of 4p16, and abnormalities of aortic and pulmonary valve was associated with trisomy 18.

Conclusion: Molecular autopsy is a significant tool for the characterization of the basis of cardiac valve defect and also become vital for an accurate genetic counseling.

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P05.21A

Somatic DNA methylation and copy number landscape of coronary artery disease patients

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DNA methylation and copy number variations (CNVs) of affected and healthy vascular tissues of patients with atherosclerosis had not been investigated in detail. The Illumina HumanMethylation27 BeadChip and Agilent SurePrintG3 HumanCGH+SNP 2×400K microarrays were used for DNA testing from right coronary arteries in the area of advanced atherosclerotic plaques and atherosclerotic-resistant internal mammary arteries of six patients with coronary artery disease. The atherosclerotic plaques to compare with the healthy arteries were characterized by predominate DNA hypermethylation changes. These genes were annotated with muscle system process and positive regulation of cytosolic calcium ion concentration in Gene Ontology terms. In contrast, hypomethylated genes encode molecules belonging to different biological processes such as development, immune/inflammation responses, lipid storage, and programmed cell death. In atherosclerotic plaques the most pronounced hypomethylation was registered in 2q31.1 (HOXD4/HOXD3/MIR10B), 7p15.2 (HOXA7) and 11p11.2 (ALX4). Moreover, methylation changes at 2q31.1 in blood cells were consistently associated with smoke and ischemic stroke. We identified 90 high-confidence CNVs that were present in matched arteries studied. Gene Ontology analysis revealed enrichments in the immune/inflammation responses and olfactory transduction. Furthermore, two patients contained the gain in 10q24.31 (ERLIN1) that affected only the blood DNA but not arteries. There was not overlap between both DNA methylation and copy number changes in arteries. In conclusion, although DNA methylation differences do not appear to be linked to the copy number changes in arteries of patients with coronary heart disease both mechanisms may be important in the disease.

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P05.22B

Monogenic and complex risk factors for ischemic heart disease in Finland - Elucidating the role of severe hypercholesterolemia

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Severe hypercholesterolemia is one of the most significant risk factors for coronary artery disease (CAD), and it has a strong and complex genetic predisposition. Currently, not even patients with inherited forms of severe hypercholesterolemia are adequately identified in Finland, and only a part of their mutation load is known.

To unravel the underlying genetic architecture, we screened 206 individuals with severe hypercholesterolemia (LDL-cholesterol >= 5mmol/l) in the prospective GeneRISK study, encompassing 7,328 subjects, aged 45-64 years, from Southern Finland. Exome-sequencing identified a novel, likely causal mutation in *LDLR* (R574L), but surprisingly we didn't identify any of the hypercholesterolemia-associated *LDLR*-mutations previously shown to be enriched in Finland. Neither did we identify potentially causal mutations in *APOB* or *PCSK9*. Polygenic modeling (106 LDL-cholesterol-associated SNPs) explained 17% of the LDL-cholesterol variation in the cohort but suggested only slight clustering of polygenes with severe hypercholesterolemia.

Evaluating the use of lipid-lowering medication in the GeneRISK-cohort, we found that only 4.3% of individuals with severe hypercholesterolemia were on lipid-lowering treatment at baseline. This is considerably less than the 10.1% that were treated in the full cohort. Our preliminary data further show that at follow-up 1.5 years later, lipid-lowering medication had been initiated only to an additional 3.3% of the individuals with severe hypercholesterolemia. Given that patients with hypercholesterolemia are poorly identified and treated, there is an unmet need not only to further characterize causal variants underlying severe

hypercholesterolemia but also to raise awareness among caretakers to take action to reduce the disease burden.

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P05.23C

Polygenic hyperlipidemias and coronary artery disease risk

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Hyperlipidemia, particularly increased LDL-cholesterol (LDL-C) or triglycerides (TGs), is a treatable risk factor for coronary artery disease (CAD). In addition to high-penetrant mutations in genes like *LDLR*, *APOB* and *PCSK9*, hyperlipidemias can be a consequence of polygenic burden. Whereas monogenic familial hypercholesterolemia (FH) increases CAD risk considerably due to high lifelong exposure to LDL-C, it is unclear whether a high polygenic load of LDL-C or TG-increasing variants increases CAD risk.

In this prospective cohort study, we constructed incident CAD events (n=970) from the genotyped FINRISK population cohort (n=20499) linked with healthcare registries. We tested if high (>90th percentile) polygenic scores for LDL-C or TG increased CAD risk.

Finnish *LDLR* FH mutations increased LDL-C 3.5 mmol/l on average, and CAD risk considerably (HR 3.67 [1.18-11.43]). In contrast, high LDL-C score increased LDL-C 0.8 mmol/l and CAD risk only marginally (HR 1.22 [1.00-1.49]).

No monogenic large-effect TG-increasing variants were present. High TG score, however, increased TG 0.6 mmol/l on average, and CAD risk significantly (HR 1.26 [1.04-1.54]). Comparing hypertriglyceridemic individuals (TG >2.5 mmol/l) to individuals with TG <1.7 mmol/l, those with high TG score had higher CAD risk (HR 2.11 [1.58-2.81]) than those without high TG score (HR 1.61 [1.33-1.94]) despite comparable TG levels (p=0.022). Only 5.2% of the hypertriglyceridemic individuals received fibrate treatment.

The CAD risk associated with a high polygenic load of LDL-C or TG-increasing variants depends directly on their effect on lipid levels. The clinical utility of polygenic scores needs further study.

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P05.24D

Refinement of coronary artery disease risk assessment by genomic information

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Introduction: Current cardiovascular disease (CVD) risk assessment relies on clinical risk scores, which fail to identify a large proportion of individuals who develop CVD. Novel biomarkers may complement such scores. Genomic information has improved risk estimation in several prospective cohort studies and is therefore the most promising candidate to enhance clinical risk assessment.

Materials and Methods: We established a novel cohort of 7439 coronary artery disease (CAD)-free 45-65-year-old individuals from southern Finland. We estimated their CAD risk using 1) an established national clinical risk score, and 2) the clinical score combined with a genetic risk score (GRS) for CAD. We returned both estimates to the participants. We aimed to 1) evaluate the CAD risk spectrum of the cohort, 2) assess how combining the GRS

to the clinical score refines risk estimation, and 3) identify high-risk individuals with actionable clinical characteristics.

Results: Genotyped participants numbered 5125. Those with high CAD risk (10-year risk ≥10%) increased from 406 (7.9%) to 452 (8.8%) with the combined score; 113 were reclassified to a lower risk category and 159 to the high-risk category. The combined score refined CAD risk clinically meaningfully in 950 (18.5%) participants. Of the 452 high-risk participants based on the combined score, 197 (44%) smoked, 165 (37%) were obese, 80 (18%) had diabetes, 310 (69%) had LDL-C >3 mmol/l, 98 (22%) used statins and 205 (45%) used antihypertensives.

Conclusions: Incorporating the CAD GRS to the clinical risk score considerably refined CAD risk estimation. High-risk individuals presented multiple risk factors and intervention opportunities.

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P05.25A

A novel candidate frameshift mutation for Catecholaminergic Polymorphic Ventricular Tachycardia

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Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a cardiac disorder which characterized by arrhythmias, sudden cardiac arrest after physical activity or emotional stress which could result from *CASQ2* gene mutations which follow autosomal recessive pattern. *CASQ2* encodes the protein Calsequestrin-2 which is a high-capacity calcium-binding protein acting as an internal calcium store in muscle. Therefore, it plays a pivotal role in excitation-contraction coupling which regulates the rate of heart beats. In this study, we identified a novel mutation on *CASQ2* (NM_001232.3) gene in two, non-related patients with overlapping symptoms such as repetitive syncope and polymorphic ventricular tachycardia. Molecular analysis of the 2 patients' by next generation sequencing showed a homozygous splice variant p.L79X (c.237delT) which is not currently present in clinical databases. We evaluated this novel variant as a likely pathogenic variant because of its potential nonsense effect. In vivo functional studies at transcriptional and translational level are underway to demonstrate the causative role of this mutation in pathogenesis of CPVT.

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P05.27C

Variation in the *LPL* gene, low density lipoprotein-cholesterol lowering alleles and risk of coronary disease and type 2 diabetes

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Background: Several pharmacological enhancers of lipoprotein lipase (LPL) are in preclinical/early-clinical development for dyslipidemia, but it is unknown if they will reduce cardio-metabolic disease risk when added to existing lipid-lowering drugs. Human genetics can be used to study if genetic-differences in LPL-mediated lipolysis contribute to these disease independent of pathways targeted by existing drugs.

Methods: Using individual-level genetic data from 390,470 individuals and a "factorial" design, we investigated the independent and combined consequences on coronary disease and type 2 diabetes risk of triglyceride-lowering *LPL*-alleles and LDL-C-lowering alleles at different genes, including those encoding targets of current LDL-C-lowering therapy (*HMGCR*, *NPC1L1* and *PCSK9*).

Results: People carrying a higher than median load of both triglyceride-lowering *LPL*-alleles and LDL-C lowering alleles had an odds ratio (OR) for coronary disease of 0.73 compared to people below the median of both exposures (95% confidence interval [CI], 0.70 to 0.76; $p = 2.8\times10^{-52}$), which was a greater effect than that observed in people with higher than median load of either exposure. Triglyceride-lowering *LPL*-alleles were strongly associated with lower diabetes risk (odds ratio per standard deviation genetically-lower triglycerides, 0.69; 95% CI, 0.62 to 0.76; $p = 2.6\times10^{-13}$). In factorial analyses, this protective association neutralized the association of LDL-C lowering alleles with a higher diabetes risk ($p_{heterogeneity}$ in effect estimates = 0.0094).

Conclusions: Triglyceride-lowering *LPL*-alleles and LDL-C-lowering genetic mechanisms have independent contributions to a lower risk of coronary disease. These findings provide human genetics evidence to support the development of agents that enhance LPL-mediated lipolysis for use in the context of LDL-C-lowering therapy.

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P05.28D

Digenic mutations of *MYH7* and *RYR2* in siblings manifesting with severe cardiac dysfunction

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Introduction: Mutations in the *Myosin heavy chain* 7 (*MYH7*) are associated with inherited cardiomyopathies. On the other hand, mutations in the *Ryanodine receptor 2 (RYR2)* are associated with fatal arrhythmias such as catecholaminergic polymorphic ventricular tachycardia. Both of them exhibit autosomal dominant patterns of inheritance.

Case Report: The proband was born at 34 weeks of gestation with a birth weight of 2452g. He presented with severe cardiac dysfunction at birth and needed strict intensive care. At 3 years-old, he had intractable epilepsy and severe neurodevelopmental impairment. An elder brother who was born at 39 weeks of gestation died shortly after birth because of cardiac failure. There were strong family history of sudden cardiac death in the father's trait. The mother manifested with heart failure after the delivery of the proband and presented with tachycardia during treadmill stress test. Whole-exome sequencing identified two variants in the siblings, a heterozygous missense variant in *MYH7* (c.728G>A) inherited from the father, and in *RYR2* (c.5428G>C), inherited from the mother. The variant in *MYH7* was reported as a pathogenic allele. The variant in *RYR2* was considered likely to be pathogenic since PHRD-like scaled Combined Annotation-Dependent Depletion (CADD) score suggested deleteriousness (25.9).

Conclusions: We report sibling cases manifesting with severe cardiac dysfunction possibly due to digenic *MYH7* and *RYR2* mutations. Digenic mutations may cause severer clinical manifestation than that caused by each mutation.

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P05.29A

Increasing the number of genes in the genetic screening of dilated cardiomyopathy: is more actually more?

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Background: Dilated cardiomyopathy (DCM) is characterized by systolic dysfunction with dilatation of the left ventricle and/or right ventricle. The Dutch Society for Clinical Genetic Laboratory Diagnostics (VKGL) previously recommended genetic testing in DCM patients using a core panel of 47 cardiomyopathy-associated genes (including *TTN*, *MYH7*, *PLN and LMNA*). However, DCM is a complex multifactorial disorder involving multiple genes. Other genes are probably involved in the pathogenesis. Therefore, a panel containing 347 genes involved in cardiac disorders was composed.

Aims: To determine the yield of genetic variants in gene panel testing involving 347 heart related genes in 100 genetically unsolved DCM patients in a Dutch population.

Methods: 100 DCM patients without (likely) pathogenic mutations in the 47 core cardiomyopathy genes were offered genetic analysis through whole exome sequencing using a targeted filter consisting of 347 heart related genes.

Results: In 51 patients, genetic analysis of the 347-heart gene panel has been completed. Variants of unknown significance, likely pathogenic or pathogenic variants were detected in 15 (29%), 3 (6%) and 0 (0%) patients respectively. So far, likely pathogenic variants were identified in *ANK2*, *KCNQ1* and *FLNC*. The gene with the highest yield is *FLNC*. Mutations in *FLNC* are associated with myopathy and cardiomyopathies. *ANK2* and *KCNQ1* are associated with inherited primary arrhythmias.

Conclusions: It is likely that *FLNC* has a major role in the pathogenesis of DCM. Therefore, *FLNC* should be part of the core panel of cardiomyopathy genes. Furthermore, variants in primary arrhythmia genes might explain a small number of patients.

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P05.30B

A targeted 18 gene panel approach detects a likely genetic cause in 25% of Scottish Dilated Cardiomyopathy patients

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Dilated cardiomyopathy (DCM) is aetiologically heterogeneous, and up to 35% of cases are found to be familial after investigation of first degree relatives. Amongst those with genetic DCM, truncating mutations in TTN are thought to be the most common cause, being found in around 30% of familial cases in most series. In Scotland, patients referred for genetic investigation of dilated cardiomyopathy are tested using 2 gene panels, one with 13 genes encoding components of the sarcomere including TTN, and the other including 5 genes also associated with cardiac disorders with an arrhythmia phenotype (SCN5A, ABCC9, PLN, DES and LMNA). The 5 gene arrhythmia panel came into use in 2017. We report an audit of the outcome of this approach. In 2017, 106 patients with DCM were referred for genetic testing in Scotland (population ~5.4 million). Only 25% had a genetic variant likely to cause DCM, 8% being associated with truncating mutations in TTN. The majority (17%) had variants in other cardiac genes (including 5% in TNNT2, 4% in ABCC9 and 2% in MYH7, other genes contributed a smaller number to the total). This real world data from the Scottish Laboratory Consortium Molecular Diagnostic service suggests that in Scotland, TTN accounts for fewer cases than might be expected from the literature. A 25% overall detection rate nevertheless suggests that targeted gene panel testing using genes for which good genotype-phenotype data is available remains a useful way to investigate for inherited DCM.

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P05.31C

Mortality Risk associated with Truncating Founder Mutations in Titin

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Background: Truncating Titin variants (TTNtv) are a major cause of dilated cardiomyopathy (DCM), a major cause of heart failure. TTNtv are also frequently present in the general population and associated with a variable phenotype. The prognosis of asymptomatic TTNtv carriers is poorly understood. **Objectives:** To assess the clinical relevance of TTNtv by analysing TTNtv associated all-cause mortality in historical pedigrees and in present-day patients.

Methods: Haplotype analysis identified five recurrent A-band TTNtv as founder mutations. Three multigenerational pedigrees were traced back to 18th century ancestors. Using the Family Tree Mortality Ratio method, standardized mortality ratios (SMR, standardized for sex, age and calendar-period) were calculated. Similarly, SMR was calculated in parents of 128 present-day DCM patients with a TTNtv using the reverse parent-offspring method. Subgroups were compared with Poisson regression.

Results: In 20,522 person-years, overall mortality was not significantly increased in three historical pedigrees compared to the general population (SMR 1.06, 95% CI 0.95-1.18, $p = 0.162$). However, mortality was significantly increased in subjects living after 1965 (SMR 1.27, 95% CI 1.04-1.53, $p = 0.009$), and subjects aged ≥60 (SMR 1.17, 95% CI 1.01-1.35, $p = 0.02$). Mutation-specific differences in mortality were observed. Reverse parent-offspring analysis showed excess mortality (SMR 1.26, 95% CI 1.07-1.48, $p = 0.003$), driven by subjects aged ≥60.

Conclusion: The natural history of TTNtv showed a relatively mild phenotype with significant excess mortality after 1965 and in subjects aged ≥60 years. Increased mortality above 60 years was also seen in parents of present-day patients. With increasing life expectancy, TTNtv-associated mortality will likely become more prevalent.

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P05.32D

DNA methylation changes in destabilization of carotid atherosclerotic plaque

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Introduction: Destabilization of carotid atherosclerotic plaque (CAP) is known to be the cause of cerebrovascular ischemia and stroke, but epigenetic framework for this complex process is poorly investigated. Our study aimed to compare genome-wide DNA methylation profiles in cells of stable and unstable atherosclerotic plaques of carotid arteries.

Materials and Methods: CAPs were obtained from 16 patients (10 males, 6 females, aged 65 ± 6 years) and divided into histologically stable ($n = 8$) and unstable ($n = 8$) samples. CpG methylation in specimens was estimated using Infinium MethylationEPIC BeadChip (Illumina) and analyzed in R/Bioconductor.

Results: After discard of sex chromosome-mapped, polymorphic, cross-hybridized and poorly detected microarray probes, of remained 775836 sites, 1829 CpG-sites showed differential methylation ($|\Delta\beta|>0.15$) according to CAP instability ($p < 0.05$) and located commonly beyond CpG-islands. Hypomethylated 1194 sites were overrepresented genes functioning in immune cells and participating in different biological processes associated with immune response (i.e. leukocyte activation, T-cell differentiation, etc.). Other 635 sites with elevated methylation level in unstable plaques resided within genes largely involved in morphogenesis and developmental processes (i.e. cytoskeleton organization, cell adhesion, etc.). Unsupervised analysis revealed moderate correlation of the first principal components with measures of lipid core and cap of CAPs, and also with heart failure severity and ACE inhibitors intake.

Conclusions: DNA methylation changes in unstable CAPs are associated with activation of immune response and impaired remodeling of artery wall, which can reflect the underlying processes of plaque destabilization on epigenetic level. The study is supported by a grant of Russian Scientific Foundation (16-15-10150).

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P05.33A

FABP4 gene expression in epicardial adipose tissue is related to obesity associated coronary heart disease

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Objective: The fatty acid-binding protein 4 (FABP4) has been described as a biomarker for adiposity. Being expressed by both adipocytes and macrophages it takes part in intracellular lipid traffic and acts as an important adipocytokine. Increased circulating FABP4 level is associated with obesity, insulin resistance and atherosclerosis. However, little is known about FABP4 gene expression in epicardial adipose tissue (EAT) during obesity and its potential influence on coronary atherosclerosis development.

The aim of this study was to evaluate FABP4 mRNA expression in EAT in patients with obesity and coronary heart disease (CHD).

Methods: We analyzed: 1) group of 50 patients with CHD which consisted of 26 obese persons and 24 normal weight persons; 2) control group of 12 individuals without CHD which consisted of 6 obese persons and 6 normal weight persons. EAT samples were obtained from CHD patients during coronary bypass surgery and from control persons during heart valve surgery. Atherosclerosis severity was evaluated by coronary angiography. FABP4 and macrophage marker CD68 mRNA levels in EAT were determined by real-time RT-PCR.

Results: FABP4 mRNA levels were significantly reduced in obese CHD patients when compared with obese controls ($p < 0.01$). Among normal weight persons there was no difference in FABP4 expression between patients with

CHD and corresponding controls without CHD. FABP4 mRNA levels within obese subgroups were highly correlated with proinflammatory macrophage marker CD68 mRNA levels ($r = 0.602$, $p < 0.01$).

Conclusions: Our results indicate that FABP4 gene expression in EAT is associated with coronary heart disease developed on obesity background.

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P05.34B

The incidence and risk of cardiovascular disease outcomes in patients with Familial Hypercholesterolaemia: a matched survival analysis using a large UK primary care prospective cohort

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Introduction: Heterozygous familial hypercholesterolemia (FH) is the most common inherited cause of raised LDL-cholesterol and is associated with an increased risk of coronary heart disease (CHD). This study aimed to determine if FH is associated with other cardiovascular diseases (CVD) including transient ischaemic attack (TIA), stroke and peripheral vascular disease (PVD).

Material and methods: Using routine primary care data from the UK Clinical Practice Research Datalink (CPRD), we used primary care disease codes to select subjects with clinical FH diagnoses who were free from CVD at baseline, and matched them with population controls according to age, sex and general practice. Cox proportional hazards regression models stratified on the matched pairs, were used to determine hazard ratios (HR) for CVD (up to 10 years follow-up) between FH patients and their matched controls.

Results: The incidence rates of CVD in patients with FH ($n = 14073$; 4,481 CVD events) and in the controls ($n = 42{,}130$; 1,767 CVD events) were 25.8 and 3.1 per 1000 person-years respectively. Compared with controls, the hazard ratio for CVD among those with clinical FH was 8.87 (95% CI: 8.31-9.47, $p < 0.0001$). The hazard ratios for coronary heart disease (CHD), TIA/stroke and PVD were 10.34 (95% CI: 9.56-11.17; $p < 0.0001$), 6.51 (95% CI: 5.65-7.51; $p < 0.0001$) and 6.93 (95% CI: 5.89-8.16; $p < 0.0001$) respectively. These hazard ratios remained significant after adjusting for known CVD risk factors.

Conclusion: Individuals with clinical FH have significantly higher risk of coronary heart disease, stroke/transient ischaemic attack and peripheral vascular disease over time.

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P05.35C

Improving identification of familial hypercholesterolaemia in primary care using FAMCAT (Familial Hypercholesterolaemia Case Ascertainment Tool): validation in a large population database

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Introduction: Familial Hypercholesterolaemia (FH) is a common inherited cause of raised cholesterol. However, over 80% of people with FH are still not identified, leading to many avoidable heart attacks and early deaths. This study assessed the validity of a new FH case-finding algorithm (FAMCAT), intended for application to patients' primary health care records, in a large population cohort.

Material and Methods: Analysis of 747,194 patients' data from QRESEARCH, a UK primary care database, was conducted by applying FAMCAT regression equations to predict probability of FH. There were 1,397 patients diagnosed with definite FH. Prediction accuracy of FAMCAT, determined by the area under the receiver operating characteristics curve (AUC), was compared to use of established Simon-Broome and Dutch Lipid Clinic criteria for FH.

Results: FAMCAT resulted in an overall AUC of 0.805 (95% confidence interval [CI] 0.793 - 0.817), performing significantly better in identifying FH than Simon-Broome (SB) criteria (0.692, 95% CI 0.680 - 0.705) or Dutch Lipid Clinic (DLC) criteria (0.716, 95% CI 0.703 - 0.729). Prediction of FH by minority ethnic group resulted in AUCs ranging from 0.755 (95% CI 0.626 - 0.885) for Asian/Asian British to 0.871 (95% CI 0.808 - 0.934) for Black/Black British/African.

Conclusions: The FAMCAT algorithm offers significant improvement for identifying FH compared to DLC or SB criteria in UK primary care practice. FAMCAT may require recalibration in, and for use other international and ethnically diverse population groups. Further research assessing the clinical utility of FAMCAT with genetic test diagnosis is recommended.

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P05.36D
Genetics, lifestyle and cholesterol in young women

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Introduction: Low-density lipoprotein cholesterol (LDL-c), a causal risk factor for atherosclerosis, is mostly studied upon clinical events. Women are usually affected later in life than men and are underdiagnosed and undertreated in cardiovascular investigations. This study addresses genetic and lifestyle factors affecting LDL-c in young women.

Methods: Premenopausal women with LDL-c ≤ 1st percentile (≤ 50 mg/dl; n = 119) and ≥ 99th percentile (≥ 186 mg/dl; n = 121) were selected from a Dutch population-based cohort (Lifelines). We applied a one-step, novel NGS-based gene-panel to establish genetic origins of hypo- and hypercholesterolemia. A "healthy lifestyle score" was extracted from questionnaires.

Results: 15,7% of women with low LDL-c carried mutations causing monogenic hypocholesterolemia and 49,6% were genetically predisposed to low LDL-c based on extremely low weighted genetic risk-scores. A healthier lifestyle was not associated with low LDL-c in women without genetic predisposition. 16,8% of women with high LDL-c carried mutations causing familial hypercholesterolemia, whereas 21% were predisposed to high LDL-c based on polygenic risk-scores. Women without such genetic predisposition exhibited a significantly unfavorable lifestyle.

Conclusions: Our study demonstrates the need for early assessment of cardiovascular risk profiles in apparently healthy young women to identify those with LDL cholesterol levels above the 99th percentile for their age: 1) 17% of the cases appeared molecularly diagnosed with familial hypercholesterolemia; 2) our data indicate that an unfavorable lifestyle is significantly associated with severe hypercholesterolemia in genetically unaffected women, which may need further evaluation and advice to prevent future cardiovascular complications.

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P05.37A
Fibromuscular dysplasia: Identifying potential genetic causal variants by whole-exome sequencing

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Background: Fibromuscular dysplasia (FMD) is a noninflammatory, nonatherosclerotic disorder of medium-sized arteries. FMD leads to arterial stenosis, occlusion, aneurysms and dissection, most commonly involving renal and internal carotid arteries. Clinical manifestations commonly include hypertension, dizziness and pulsatile tinnitus.

The etiology of FMD remains unknown. Since FMD occurs in families in approximately 10%, a genetic origin seems plausible.

Aims: To identify genetic variants involved in FMD.

Methods: So far, genetic analysis through whole exome sequencing was performed in five patients with FMD. Only rare and novel truncating, missense and deletion variants predicted to be conserved and deleterious are considered to be of interest.

Results: In one patient, we identified a missense variant in *COL4A1*, a nonsense variant in *COL4A2* and an 85kb deletion in *ACTA2*. In two other patients, a missense variant in JAG1 and a missense variant in *COL4A2* were identified respectively.

The collagen IV network seems to be critical for structural integrity in the basement membrane. *COL4A1* and *COL4A2* mutations are associated with multisystem disorders with abnormalities in the vasculature and other organs.

Mutations in *JAG1* causes Alagille syndrome in which vascular anomalies including bilateral renal artery stenosis leading to hypertension and internal carotid artery aneurysms have been reported.

Literature reports one mutation in *ACTA2* causing dilatation of proximal internal carotid artery, occlusive disease of terminal internal carotid artery, and abnormally straight course of intracranial arteries.

Conclusions: FMD is likely to be a multifactorial disorder, however vascular and connective tissue genes might be involved in the pathogenesis of FMD.

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P05.38B

Discovery and fine-mapping of type 2 diabetes susceptibility loci in diverse populations using more than a million individuals

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To discover type 2 diabetes (T2D) loci and enhance fine-mapping resolution, we conducted the largest meta-analysis of genome-wide association studies of the disease to date by aggregating 171,262 cases and 1,075,072 controls from diverse populations (45% non-European ancestry). We identified 208 loci at genome-wide significance ($p<5x10^{-8}$), including 41 mapping outside regions previously implicated in T2D (accounting for those discovered in European-specific component of this study). Across these loci, conditional analyses revealed a total of 342 distinct signals of association (locus-wide significance, $p<10^{-5}$). We observed strong evidence of heterogeneity in allelic effects on T2D ($p_{HET}<1.5x10^{-4}$, Bonferroni correction) correlated with ancestry at 16% of signals, including *LEP* (rs7778167, $p_{HET}=4x10^{-25}$, East Asian specific) and multiple associations at/near *KCNQ1* and *TCF7L2* (representing ethnic-specific/-differentiated effects). T2D-associated variants showed significant enrichment (odds-ratio range 1.90-6.63; $p<0.05$) in coding exons, pancreatic islet enhancers and promoters, adipose enhancers, and binding sites for transcription factors, including NKX2.2 and FOXA2. Increased sample size, population diversity, and annotation-informed fine-mapping substantially improved localisation of potential causal variants compared with previous efforts, and highlighted 76 signals with a single variant accounting for >80% of the posterior probability of association (PPA); of these 35 signals had PPA of >99%. Clustering of signal-specific annotation enrichment highlighted distinct clades of T2D associations driven by different underlying molecular processes. These analyses represent the most comprehensive view of the genetic contribution to T2D to date and, through integration with expression quantitative trait loci in disease-relevant tissues, point to previously unreported effector genes and mediating molecular mechanisms at several loci.

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P05.39C

Copy number variation analysis in cardiac congenital septal defects

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Congenital cardiac septal defects (CCSD) are the most frequently type of congenital heart malformation, often occurs sporadically, without an obvious cause in most cases. Recent studies demonstrated the involvement of several genes variation in cardiac development process, factors that have been underestimated in the past. The aim of our study was to evaluate if the CCSD patients present copy number variations (CNVs) and establish whether the CNVs investigation should be performed in management of this patients. A number of 26 pediatric patients were enrolled. The CNVs were determined using the multiplex ligation-dependent probe amplification (MLPA) technique

and P234-A3 SALSA MLPA probemix which contain probe for GATA 3 and GATA 4 gene analysis. The MLPA analysis revealed CNVs in two patients. Both of them present a duplication located in 10p14 (GATA 3 gene, exons 4 and 5). Several patients present a high ratio for 8p23.1 (GATA 4 gene) and a low ratio for 10p14 (GATA 3 gene) but the signals were statistically insignificant. Six patients present low ratio for flanking probe (CELF2 probe, according to the manufacturer CNVs of flanking probes are unlikely to be related to the condition tested). Twenty patients received recommendation for target sequencing of different exons. Based on our results, we may consider the mentioned MLPA kit as a first step in CCSD patient's management.

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P05.41A

Next Generation Sequencing (NGS) panel revealed new candidate genes and variants in 25 Hypertrophic Cardiomyopathy patients

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Introduction: Familial Hypertrophic cardiomyopathy (HCM) is a multigenic heart condition characterized by hypertrophy of the cardiac muscle. The prevalence of HCM is estimated at 1:500 in the general population.The aim of our study was evaluation of complex genetic profile of next generation sequencing (NGS) results in Turkish patients with HCM.

Methods: We designed a targeted cardiac panel of 68 genes using NGS technology on Iontorrent PGM.

Results: Analyses of 25 HCM patients revealed a total of 54 variants in cardiac genes of which 9 (16,7%) were pathogenic, 5 (%9,2) were potentially pathogenic, 34 (63%) were variant of unknown significance (VUS) and 6 (11,1%) were likely benign. Only one of the patient (4%) had no suspicious variant in examined genes. 10 patients (40%) carried at least one pathogenic/potentially pathogenic mutations in *MYH6, MYH7, MYBPC3, TNNI3, TNNT2, DSP, DPP6, JUP, KCNQ1* and *SCN5A* genes, while other 14 patients (56%) had at least one VUS variant. A total of 12 novel variants were identified, which consist of 1 frameshift insertion in *JUP* gene, 1 frameshift deletion in *DPP6* gene and 9 missense variants in *DPP6, MYH7, MYH6, GJA5, NKX2-5, TMPO* and *TRDN* genes. An interesting finding is co-occurrence of dominant pathogenic/likely pathogenic variants in different genes, as in the three cases explains the complexity and severity of the observed phenotypes.

Conclusion: Identification of pathogenic/potentially pathogenic variants and novel candidate variants/genes has led to new opportunities for prevention and therapy of lethal HCM. **Acknowledgements:** The study was supported by SANTEZ Grant (0253. STZ.2013-2), Turkey.

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P05.43C

Identification of a novel homozygous loss-of-function variant in *JPH2* in two unrelated families affected by lethal Neonatal hypertrophic cardiomyopathy

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Pediatric cardiomyopathies represent a clinically and genetically heterogeneous group of disorders affecting the ventricular myocardium, with an annual incidence of ~ 1.5 per 100,000 children. They are associated with substantial morbidity and mortality: up to 40% die or undergo transplantation. Here we studied two unrelated consanguineous families from the same geographic region of Iran, with 4 children who died due to infantile cardiomyopathy. Affected offspring presented with severe hypertrophic cardiomyopathy and right atrium enlargement in utero, at birth, or in early childhood. Whole exome sequencing (WES) of DNA from the proband of the first family led to identification of a novel homozygous frameshift variant (p. Glu641*) in *JPH2*. The parents of the proband and a healthy sibling were heterozygous. Echocardiography revealed no abnormalities in the carriers. Due to unavailability of samples WES could only be carried out for the parents of the second family, however the same heterozygous frameshift variant was identified in both parents. The variant is novel and absent from population databases including gnomAD, the ethnically-matched GME variome, Iranome, and in ~1000 WES in-house control subjects from the same geographic region as the families investigated. The variant occurs in the protein C-terminal region of JPH2, just upstream of a 22-amino acid transmembrane anchor responsible for binding of the protein to the sarcoplasmic/endoplasmic reticulum, thereby potentially disrupting binding. Our findings add to the growing evidence that mutations in *JPH2* play a role in HCM; and suggest that this novel biallelic truncating mutation can give rise to severe, early-onset pediatric cardiomyopathy.

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P05.44D

Functional analysis of six novel LDLR mutations in the Argentinian population

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Introduction: Mutations in the gene encoding the low-density lipoprotein receptor (LDLR) are involved in the molecular etiology of autosomal dominant familial hypercholesterolemia (FH), an hereditary condition associated with coronary heart disease and the risk of premature death. Based on FH prevalence among Caucasians, it is expected that >80,000 cases are present in Argentina, less than 1% of which have been identified so far. Previously, in a cohort of Argentinian FH patients we detected eight unreported LDLR single nucleotide variants (SNVs). Six of them are located in the coding regions of the gene and lead to an amino acid substitution. To assess the pathogenicity of each SNV, we performed a functional investigation of the corresponding variant of the receptor proteins.

Materials and Methods: Binding, uptake, and degradation of 125-I-labeled LDL by cultured CHO cells expressing wild-type and mutant LDLR proteins were compared in the presence and absence of an excess of unlabelled LDL. Levels of LDLR mRNA expression were determined by qRT-PCR 48 h after transfection with plasmids.

Results: Four out of the six LDLR mutants showed a markedly reduced in vitro receptor activity, suggesting their role in the pathogenesis of FH.

Conclusions: According to in silico predictions and conservation analysis in multiple species, two of the six identified LDLR variants are likely benign SNVs. The remaining four variants can be categorized as "pathogenic variants" that expand the spectrum of FH-linked LDLR mutations in the Argentinian population.

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P05.45A

Exome sequencing in Russian families with noncompaction cardiomyopathy

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Introduction: Left ventricular noncompaction cardiomyopathy (LVNC), a relatively rare cardiomyopathy, is characterized by a high incidence of serious complications and early death. Over 60 genes associated with the development

of family cases of LVNC are described, in most cases the inheritance type is autosomal dominant. However, causal variants in these genes are detected in less than 50% of families. The search for new genes is actual.

Materials and Methods: We formed the cohort of patients with LVNC and their relatives of 1 and 2 degrees of kinship. The enrollment of participants was done by cascade and reverse-cascade methods in the heart failure center. Probands should have met echocardiographic and cardiac MRI criteria for noncompaction. Molecular testing was performed using exome sequencing for members of 14 families (43 participants). We searched for pathogenic and probably pathogenic variants in 66 genes associated with LVNC and additionally in 122 genes associated with other cardiomyopathies.

Results: In 8 out of 14 families pathogenic or probably pathogenic variants of the nucleotide sequence were identified. Only 4 variants were found in genes associated with LVNC - 2 pathogenic variants in *TTN* and 2 probably pathogenic in *MYBPC3* and *TPM1*. One pathogenic variant was found in *VCL* and 3 probably pathogenic variants were found in *DES, TBX1* and *FHOD3* associated with other cardiomyopathies.

Conclusions: The data obtained may indicate the detection of 4 new genes *(DES, VCL, TBX1, FHOD3*) associated with LVNC. The reported study was funded by RFBR according to the research project #17-04-00521.

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P05.46B
Long QT syndrome mutation spectrum in Russians

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Mutations in more than 16 genes can cause long QT syndrome (LQT). Detection of variants in these genes is necessary for diagnostics, targeted gene therapy, behavioral management, family screening and prophylaxis of sudden cardiac death. We report frequencies of disease-causing variants from 73 patients with LQT. For all patients we did exome sequencing using Agilent Focused Exome enrichment panel and Illumina HiSeq2500. For variant calling and pathogenicity scoring we followed ACMG guidelines. We also confirmed all mutations by Sanger sequencing. No disease-causing mutations were identified in 14 cases suggesting new genes might be involved in pathogenesis. 59 patients had 91 disease-causing variants in LQT and cardiac arrhythmias associated genes. 27 patients had more than one variant in those genes. We discovered 22 new variants, that were not reported previously in dbNSFP, Clinvar, OMIM, HGMD, 1000Genomes and ExAC . In almost half cases (46) we discovered pathogenic variants in KCNE1, KCNH2, KCNQ1 or SCN5A genes. In remaining cases (45) we found pathogenic variants in 18 other genes, including 7 variants in ANK2, which are rarely reported in LQT patients.This work was funded by Fundamental Scientific Research Program of the Russian Academy of Sciences for 2013-2020.

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P05.47C
Lymphedema distichiasis syndrome: dominant FOXC2 mutations causing protein aggregation and loss of transcriptional activity

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Introduction: Forkhead transcription factor FOXC2 is essential for the correct development and maintenance of lymphatic system. Mutations in the *FOXC2* gene are associated with primary lymphedema-distichiasis syndrome (LD), a congenital autosomal dominant disorder that causes a dysfunction of the lymphatic vessels. It has been demonstrated that *FOXC2* variations can either reduce or increase protein function. To clarify the molecular mechanism through which *FOXC2* mutations can affect transcriptional activity, we analyzed two missense mutations (p.L80F and p.R121H), two frameshift variations (p.H199Pfs264* and p.M276Dfs186*) and one nonsense mutation (p.Y109*), previously identified in LD patients.

Material and Methods: To investigate subcellular localization, HeLa cells have been transfected with *FOXC2* mutant plasmids, expressing FOXC2 with GFP at the N-terminus, and immunofluorescence analysis has been performed. Subsequently, transactivation activity has been

evaluated by a Luciferase assay. Finally, the effects of missense and nonsense mutations on FOXC2 protein structure have been evaluate using bioinformatics tools.

Results: p.L80F, p.R121H, p.H199Pfs264* and p.M276Dfs186* were able to correctly localize in the nucleus, but they produced FOXC2 protein aggregates characterized by partial or total loss of transcriptional activity. Moreover, p.H199Pfs264* and p.M276Dfs186 protein aggregation involved also chromatin structure. The p.Y109* also caused the formation of protein aggregates, even though it was mainly localized in the cytoplasm. Finally, the analysis of FOXC2 protein structure revealed that missense and nonsense mutations dramatically modified the DNA binding-site conformation.

Conclusions: In Lymphedema-distichiasis, FOXC2 mislocalization, structure modification and nuclear or cytoplasmic aggregates formation can be considered the main cause of loss of protein function

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P05.48D

Induction of angiogenesis by M13 bacteriophage-RGD nanofibrous surfaces on human umbilical vein endothelial cells

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Introduction: The ability to create, remodeling and regulate the human blood system holds wide range of medical utilization and carries possible therapeutic effects on patients with variety of cardiovascular diseases. Commonly, Clinical use of autologous vessels has been reported; However, transplant rejection unfortunately occurs in most patients. Likewise, biomaterial tissue engineering on vascular replacement devices has been recently improved.

Materials and Method: In the following study, comparing the ability of M13 Bacteriophage, M13 bacteriophage-RGD and Gelatin surfaces to enhance the angiogenic potential of human umbilical vein endothelial cells (HUVECs) was investigated, The response of HUVECs cells to surfaces were studied through cell apoptosis, cell proliferation, growth factors secretion (VEGF), and angiogenic-endogenic-associated genes (Matrix metallopeptidase 9 (MMP9), Endothelial nitric oxide synthase eNOS). Real Time-PCR, ELISA, Nitric Oxide assay and Zymography was operated on samples. After 2 days of cell seeding, Gene and Protein expression data were analyzed and compared by two-way analysis of variance.

Result: Our results show that M13 phage-RGD appeared Non-cytotoxic to the cells. Furthermore, these result was associated with a markedly increase on cell proliferation and migration. M13 phage-RGD characterized a strong increase in MMP-9, eNOS and VEGF-A mRNAs as well as leading to higher expression level of VEGF-A protein, MMP-9 activity and NO release in HUVECs in comparison to other surfaces.

Conclusion: Beneficial effect of M13 phage-RGD propose these nanostructures as an advantageous and potential biomaterial in order to promote angiogenesis, which are becoming feasible therapeutic applications for variety of ailments.

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P05.50B

The role of genetic variation in phenotype variability and response to treatment in Marfan syndrome

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In a large cohort of 300 clinically diagnosed MFS patients, we addressed the following questions: Which proportion of classic MFS patients has an *FBN1* mutation? Are there genotype-phenotype correlations? Does the nature of the *FBN1* mutation predict cardiovascular treatment outcome? Next-generation targeted resequencing of *FBN1* and related genes was followed by deletion/duplication testing. Pathogenic *FBN1* mutation was identified in 94% of patients (including 15 del/dups). Three percent of MFS patients had non-FBN1 mutations. A similar small fraction had no causal variant but was clinically indistinguishable from the *FBN1* mutation positives. We confirm prior literature that mutations creating or deleting cysteines are significantly associated with lens dislocation ($p = 10\text{-}7$). Premature termination codons are more often associated with skeletal findings, but not with cardiovascular severity. We did not observe a correlation between mutations in the middle region of *FBN1* and phenotypical severity. Mutations at the C- and N-terminal (exons 1-16/60-66) end tend to lead to a milder cardiovascular phenotype ($p = 0.047$). We observed no difference in aortic root size or progression nor in treatment outcome comparing dominant negative with haploinsufficient mutations. No effect of mutation location on treatment outcome could be detected. Patients that started treatment before age 10 had less aortic growth than older patients, irrespective of treatment type. A comprehensive molecular analysis identifies an *FBN1* mutation in

the overwhelming majority of MFS patients. With one exception, no major genotype-phenotype correlations can be identified. Importantly, early start of treatment has better outcome with regards to aortic root growth.

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P05.51C

Next generation sequencing in the diagnosis of Marfan syndrome and related disorders: an efficient global approach in the SNV/CNV detection

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Introduction: The Genetic Laboratory in Bichat-Hospital (Paris) receives each year samples for approximatively 600 probands suspected for Marfan Syndrome (MFS) or Related Disorders (RD) nationwide. Genetic heterogeneity is high and new genes are regularly discovered. Initially, the strategy for molecular diagnosis was based on successive Sanger sequencing of the main genes, associated with MLPA analysis. The objective was to evaluate on these probands a NGS capture panel strategy, targeting 25 known disease causing genes.

Material and Methods: Sequencing was carried out on MiSeq (Illumina). Bio-informatical analysis was performed on CLC Genomics Workbench software; Single Nucleotide Variants (SNV) annotation was completed with a Python script. Copy Number Variation (CNV) were sought through comparison of coverage depths, standardized for each amplicon to those of a group of controls.

Results: To date, more than 600 probands have been sequenced on this capture panel; a potentially pathogenic variant was found in approximatively 200 patients. This led us to identify the disease causing variation in new genes in families in which the most frequent genes had already been excluded. This global approach enabled us to expand the phenotypic spectrum of pathogenic variants in some genes. Moreover, several pathogenic CNV have been pinpointed, either in some genes that were not screened for CNV before.

Conclusions: NGS technologies enable us to have a unique strategy in the molecular diagnosis of MFS and RD. The chosen technology is reliable for SNV and CNV detection and flexible since the targeted genes can easily be adapted as scientific knowledge develops.

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P05.52D

Genotype-phenotype correlations in Marfan-syndrome for the prediction of severe cardiovascular manifestations

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Introduction: Marfan syndrome (MFS) is an autosomal-dominant, systemic connective tissue disorder, with a prevalence of ~1:5000. The most important, life-threatening complication is aortic dissection, therefore the identification and the follow-up of aortic dilatation as well as prophylactic surgery is essential. In most cases, heterozygous FBN1 gene mutations are responsible for the disease, leading to the reduction (haploinsufficiency=HI) or to the abnormal structure (dominant negative type mutation=DN) of the fibrillin-1 protein.

Aims: We examined the association of FBN1 sequence variations with cardiovascular (CV) involvement.

Methods: Phenotypic evaluation was carried out according to the revised Ghent nosology, while molecular genetic analysis of the FBN1 gene was performed using next-generation sequencing and Sanger-sequencing techniques in 35 MFS patients.

Results: Pathogenic mutations were identified in 20 cases (57%; DN-type: 9 missense and 11 HI-type: 5 nonsense, 2 frameshift, 3 splice mutations and 1 large deletion affecting exons 2-4). There was no significant difference between the occurrence of major CV symptoms (aortic dilatation and/or dissection) in the patient cohorts with or without FBN1 mutations (60% vs. 85%, $p = 0.13$). There was no significant difference between the effect of DN and HI mutations on the major CV traits (66% vs. 100%, $p = 0.07$). Among patients with non-cysteine missense mutations, CV symptoms occurred with significantly lower probability compared to DN mutations affecting cysteine residues or HI mutations (25% vs. 100%, $p < 0.01$).

Conclusion: The identification of disease-causing FBN1 mutations in MFS patients can improve the accuracy of the risk estimation of aortic disorders and planning prophylactic aortic root reconstruction surgeries.

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P05.53A

MicroRNA expression in the setting of stability of human atherosclerotic lesion

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Background: MicroRNAs have been reported to participate in atherogenesis and to serve as markers of atherosclerosis, but the involvement of microRNAs in destabilization of atherosclerotic plaque is under-investigated. Objective: Identification of microRNAs differentially expressed in the cells of carotid atherosclerotic plaques depending on the degree of its stability.

Material and Methods: The samples of carotid atherosclerotic plaque were obtained from 14 patients. After histological analysis, all samples were divided into stable (n = 8) and unstable (n = 6) atherosclerotic plaques. MicroRNA expression was analyzed by massive parallel sequencing (miRNA-seq).

Results and Discussion: We found 161 microRNAs to be differentially expressed between stable and unstable atherosclerotic plaques. After disposal of microRNAs with low expression level (<10 CPM) in all samples and having a strong correlation with clinical features (including drug intake and other diseases), only 9 microRNAs showed significant differences. Six of them (let-7b-5p, miR-7704, miR-328-3p, miR-1291, miR-370-3p, miR-148a-5p) showed increased level of expression, and three microRNAs (miR-101-3p, miR-199a-5p, miR-125a-5p) were hypoexpressed in unstable plaques. These results are well explained by microRNA-associated pathological processes described in atherosclerosis. Principal component and cluster analyses showed similar results. Two first principal components accounted for 99% of data variability and showed a fine division of samples by stability, which can indirectly indicate that the selected microRNAs characterize the development of the pathological process.

Conclusions: Of 161 microRNAs identified to be differentially expressed between stable and unstable carotid plaques, nine microRNAs can reliably be associated with destabilization of atherosclerotic lesion. The study is supported by RSF №16-15-10150.

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P05.54B

Genotype-phenoype correlation in probands with three recurrent (founder) mutations in the MYH7 gene (V964L, M982T and G1057S)

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Aims: MYH7 is one of the most frequently mutated genes in different types of cardiomyopathies. We aimed to gain more insight in the genotype-phenotype correlation of three recurrent mutations (two founder mutations and one hotspot mutation) in the S2 structural subdomain of the MYH7 gene (V964L, M982T and G1057S). Moreover, we aimed to reach national consensus on the interpretation and classification of these three mutations.

Methods: We retrospectively collected information on clinical and cardiologic characteristics as well as additional risk factors possibly influencing the phenotype of 55 probands with one of the three recurrent mutations V964L (n = 30), M982T (n = 16) and G1057S (n = 9) in MYH7. If available, results of segregation analysis in (first degree) family members were retrieved.

Results: Probands with one of the mutations V964L and M982T displayed varying types of CMP whereas probands with the G1057S mutation all had a diagnosis of HCM. In the majority of these probands additional risk factors (most often hypertension) and/or a mutation in a second

sarcomeric gene were identified. The limited amount of segregation data are inclonclusive at this point in time.

MYH7 mutation	V964L (n = 30)	M982T (n = 16)	G1057S (n = 9)
Second sarcomeric gene mutation	7 (23%)	5 (31%)	1 (11%)
Hypertension	5 (17%)	4 (25%)	5 (56%)
Second mutation AND hypertension	4 (13%)	–	–
Other risk factors	1 (3%)	2 (13%)	–
No risk factors	13 (43%)	5 (31%)	3 (33%)

Conclusion: The high number of additional risk factors (mainly second sarcomeric gene mutations and/or hypertension) in probands with the mutations V964L, M982T and G1057S in MYH7 supports the notion that these variants may be not be causal by themselves, but are possibly modifiers of disease.

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P05.55C

Genetic variants lowering the levels of coagulation factor X are protective against myocardial infarction

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Background: Coagulation factor X (FX) plays a pivotal role in the clotting process, being responsible for thrombin generation; emerging evidence suggests its involvement also in non-hemostatic processes, including inflammation. Recent data demonstrated that FX inhibition reduces the risk of recurrent atherothrombotic events in patients with acute coronary syndrome.

Methods: Whole-exome sequencing (WES) and genome-wide genotyping data, derived from an Italian cohort of ~1,600 early-onset myocardial infarction (MI) cases and 1,600 controls, were analyzed to test whether rare/common variants affecting the expression levels of the *F10* gene, encoding FX, are associated with MI risk.

Results: Bioinformatics analyses, measurements of plasma FX levels, and data mining in publicly-available databases were used to assess the pathogenicity of the rare variants identified by WES in the *F10* gene. An enrichment in the burden of potentially-deleterious variants was observed among controls (OR=0.47, P=0.02), thus highlighting a reduced MI risk in subjects carrying one *F10* inactivating mutation. Moreover, the case-control association analysis performed on common polymorphisms known to influence *F10* levels (GTEx data) evidenced a significant protective effect of the rs4907485T>G variant. In particular, the GG genotype associated with lowered *F10* expression in the GTEx database was enriched among controls (37.7% vs 33.2%; OR=0.92, CI=0.84-1.02, P=0.0093), again suggesting that lowered *F10* levels are protective against the disease.

Conclusion: We showed for the first time that variants lowering FX levels are associated with reduced MI risk, thus supporting, on a genetic basis, clinical trials' results showing that FX inhibition is beneficial for the treatment/prevention of atherothrombotic events.

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P05.56D

Family study shows the phenotypic and genetic spectrum of familial noncompaction cardiomyopathy

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Introduction: Noncompaction cardiomyopathy (NCCM) is a heterogeneous cardiomyopathy characterized by excessive

trabeculation of the left ventricle (LV). Patients diagnosed with NCCM, may also have a dilated left ventricle or a hypertrophic septum.

Purpose: Indentify if index cases and affected relatives had similar NCCM phenotypes, and if cardiac phenotypes were linked to genotypes.

Methods: A retrospective family study assessed the familial cardiac phenotype and genotype in 114 families of consecutively diagnosed cases with NCCM.

Results: Family screening identified 109 relatives with a cardiomyopathy from 58 (51%) families. More affected relatives were identified in families with a mutation (29% vs 15%; $p < 0.001$). 33% of the mutation carriers did not have a cardiomyopathy. Clinical features in relatives were less severe than in index cases, 51% of the relatives diagnosed with NCCM were asymptomatic ($p < 0.001$). The non-dilated phenotype of NCCM segregated in families of index NCCM cases with normal LV dimensions, and the dilated NCCM phenotype predicted risk of having a dilated LV for relatives ($p = 0.002$). Dilated NCCM were older ($p = -0.017$), had more often LV systolic dysfunction ($p < 0.001$), RV systolic dysfunction ($p = 0.012$), MACE ($p = 0.007$) and was associated with mutations in the tail of MYH7 ($p < 0.001$). Hypertrophic NCCM was associated with *MYBPC3* mutations and HCM in the family ($p = 0.006$). HCM or DCM without trabeculation in the family increased risk for MACE in NCCM patients ($p = 0.05$).

Conclusion: From the phenotype of the index case, the familial mutation and the phenotypes of relatives, predictions can be made on risk for phenotype and outcome in relatives.

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Splicing mechanism evaluation of NGS detected variants using hybrid minigenes

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Pulmonary Arterial Hypertension (PAH) is a rare and progressive disease characterized by vascular remodeling and the increase of vascular resistance that leads to right heart failure and, ultimately, death. PAH genetic basis has been slowly uncovered during the last decades. After screening with a sequencing panel for PAH related genes, several mutations were detected in the *ATP-Binding Cassette transporter subfamily C member 8* (*ABCC8*) (Exon 3 c. G298A p.E100K, Exon3 c.2694+1G>A, Exon 11 c. C1643T p.T548M, Exon 26 c.3288_3289del pL1096fs, Exon 27 c.G3384A p.D1132N), a gene widely related to congenital hyperinsulinism. DNA fragments, wild type and mutated sequence, were cloned into the pSPL3 vector. After PCR and Sanger sequencing to confirm the fragment insertion into the plasmid, pSPL3 was transfected into the COS-7 cell line by triplicate. 48 hours' post-transfection, RNA was isolated and cDNA was generated using RT-PCR. Lastly, a high fidelity polymerase was used to perform a PCR using primers surrounding vector's exon trap, the product was analyzed via electrophoresis and the bands of interest were sequenced. *In silico* analysis predicted a moderate ANNOVAR effect in 3/5 mutations, high in 1/5 and no effect in the last one. Our experimental results showed an altered splicing pattern in 1/5 mutations checked. Sequencing is in progress to confirm the differential pattern. In conclusion, minigene assay is a simple and effective method to check for splicing alterations and pathogenicity of the variants detected. Nonetheless, analysis of patient´s RNA will be definitive to confirm our results.

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P05.58B

Landscape of mutations found by gene panel routine sequencing for pulmonary hypertension

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Introduction: Since the discovery of BMPR2 mutations in 2000, mutations in several other genes, related or not to BMPR2 signaling, have been discovered, making pulmonary hypertension (PH) a heterogenous genetic disease. Here, we sought to determine the genetic architecture of PH gene mutations in the french PH cohort.

Methods: We analyzed 227 patients with a clinical diagnosis of pulmonary arterial hypertension (PAH) or pulmonary veno-occlusive disease (PVOD), by a targeted capture panel including known PH genes.

Results: Following clinical examination, 159 subjects were classified as idiopathic PAH (iPAH), 11 as familial PAH (fPAH), 7 as drugs or toxin induced PAH and 50 as PVOD. A mutation was identified in 42 of the 227 analyzed subjects: 27 in iPAH, 7 in fPAH and 8 in PVOD. No mutation was identified in patients with drugs or toxin induced PAH.

We identified 23 mutations in *BMPR2*, 2 in *ACVRL1*, 5 in *TBX4*, 1 in *GDF2*, 1 in *SMAD9* and 9 biallelic mutations in *EIF2AK4*. No pathogenic mutation was identified in *KCNK3* or *CAV1*. However 1 variant of unknown significance was identified in each of these genes.

Conclusions: Gene panel NGS sequencing is an efficient tool to improve the knowledge of PAH genetic architecture. In our cohort, the major gene remains BMPR2, however mutations in other PH genes account for a small number of cases. Identification of these mutations allows the involvement of these genes in PH to be confirmed since only few cases were previously reported in the literature.

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P05.59C

Multi-ancestry genome-wide association meta-analysis of 293,000 individuals identifies 217 regions for the electrocardiographic PR interval

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Introduction: The electrocardiographic PR interval represents cardiac atrioventricular conduction, a critical physiological process that is associated with arrhythmias and all-cause mortality. Yet the biological determinants of the PR interval remain incompletely understood. We conducted the largest genetic association study of the PR interval to date.

Methods: We combined genome-wide association results for the PR interval from 55 studies encompassing 293,051 individuals (271,570 European, 8,173 African, 12,823 Hispanic, and 763 Asian ancestry) using fixed-effect meta-analysis. Analyses included ~12 million variants (minor allele frequency, MAF>0.1%) imputed using the 1000 Genomes Project reference panel.

Results: We identified 217 regions (152 novel) associated with PR interval exceeding genome-wide significance ($p < 5x10^{-8}$). Among novel regions, we identified 3 missense variants annotated as deleterious and/or possibly damaging in *KIAA1755*, *ARHGEF40*, and *SPSB3*; and 7 variants in high LD ($r^2>0.8$) with missense variants in *DERL3, DUSP13, DNAH11, C10orf71, ACCN4, CHPF, OBSL1* and *DALRD3*. Expression quantitative trait locus (eQTL) analysis using the GTEx portal revealed 5 variants associated with gene expression levels in right atrial appendage (*TRAK1*, *SMARCB1*, *SYNE2*, *DEK, DNAH11*). Gene Ontology enrichment analysis including only the nearest genes to both known and novel variants indicated further enrichment of biological processes involving heart and cardiac muscle tissue development with the addition of the newly identified genes.

Conclusions: Our results implicate specific genes which determine PR interval and highlight the complex polygenic nature of atrioventricular conduction. Future analyses will assess the relations between genetic determinants of the PR interval and cardiac arrhythmias.

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P05.60D

Identification of causative genes or phenotype modifiers variants associated with Pulmonary Arterial Hypertension

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Background: Pulmonary Arterial Hypertension (PAH) is a rare disease of unclear etiology that is associated with abnormally increased pulmonary pressures and chronic right heart failure. Use of whole exome sequencing (WES) has led to the discovery of gene variants linked to PAH pathobiology. The main aim of this project was to perform WES analysis of 56 unrelated PAH patients to identify gene variants potentially involved in PAH.

Material and Methods: Bioinformatic and in silico analysis was applied to WES data from patients with idiopathic, heritable and secondary induced PAH. Expression of WES candidate genes was assessed in pulmonary microvascular endothelial cells (PMVECs) from healthy donors and PAH patients. Functional gene analysis was carried via tube formation and scratch assays.

Results: A mutation in *CRIPAK* was found in a high significant percentage in PAH patients compare to controls. CRIPAK is the key protein responsible for regulation of PAK1, a major signaling mediator of VEGF responsible for promoting proliferation, migration and survival of PMVECs. PAH PMVECs lysates demonstrated significant reduction in CRIPAK protein levels along with concomitantly increased phosphoPAK1 levels. PAH PMVECs exhibit impaired tube formation and motogenic responses in culture. Thus, transfection of CRIPAK siRNA into healthy PMVECs significantly reduced angiogenic response to VEGF-A, as evidenced by reduced tube network formation and gap closure in matrigel and scratch assays, respectively.

Conclusion: WES analysis has identified *CRIPAK* as a potential modifier gene in PAH. Reduced CRIPAK could contribute to PAH by reducing endothelial viability, promoting small vessel loss and accelerating vascular remodeling.

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P05.61A

Genome-wide association analysis of recurrent myocardial infarction in UK Biobank identifies suggestive evidence for association to twenty seven loci

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Background: Despite the decline in their incidence rates, the recurrent myocardial infarction (MI) events are associated with significant morbidity, short- and long- term mortality. Relative to our understanding of risk for first events, the aetiology of recurrent MI is poorly understood.

Methods: We used UK-Biobank, a large prospective cohort of 500,000 individuals, to investigate the genetic predisposition of recurrent MI. We performed a GWAS in 3386 UK-Biobank participants admitted to hospital due to MI at least twice within a period of 28 days - 1.5 years and 8567 controls with one unique hospital record with MI diagnosis or MI hospital admissions, which occurred outside the aforementioned period.

Results: In total, 215 variants representing 27 loci reached a suggestive significance level of 10^{-5}. Among these, 17 loci have been implicated in coronary artery disease (CAD) and other cardiovascular phenotypes (eg. *KCNN2*, *KLF4*, *CACNB2*, *ADIPOR2*, *KLF5*, *PKD1L3*), known CAD risk factors (blood pressure, *CACNB2*; lipid levels, *ABHD4*), cardiac remodelling (*MAP3K5*, *SEMA3A*), and abnormalities in platelets and coagulation (*GRM7*, *KALRN*, *P2RY1*). Five of the identified genes (*CHD7*, *IST1*, *KIAA1958*, *MAP3K5*, *UBFD1*) were also found to be differentially expressed six months after a MI in 39 MI survivors (Greek Recurrent Myocardial Infarction Cohort) that had not experienced any recurrent event during that period (p-adj $\leq 10^{-5}$).

Conclusions: We identified 27 loci associated with increased risk of recurrent MI. We aim to identify

independent datasets to replicate our findings, aiming to a greater understanding of recurrent MI determinants. Supporting British Heart Foundation grant to O.G (FS/14/66/31293)

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P05.62B

Patient variation in cholesterol response and the risk of cardiovascular disease: A UK population-based cohort study

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Introduction: Statins are recommended to prevent cardiovascular disease (CVD). In many patients, statins do not achieve optimal cholesterol lowering effects due to clinical and genetic factors. This large population-based cohort study assessed LDL cholesterol response to statins and its association with future incidence of CVD.

Material and Methods: 200,225 patients (mean age of 62.8 years; 47.5% females), with at least 2 cholesterol measurements and without prior CVD at baseline, were followed from their first statin prescription date, in a population-based cohort using electronic health records from the UK Clinical Practice Research Datalink. A greater than 40% reduction in baseline cholesterol level within 24 months was classified optimal statin response in line with national guidelines. Cox regression models were used to determine hazard ratios for incident CVD events between optimal and non-optimal statin responders.

Results: A total of 97,377 (48.6%) of patients were optimal statin responders. During the follow-up, 21,973 incident CVD events occurred (10,310 in optimal responders and 11,663 in non-optimal responders). The rate of CVD was 18.8 and 16.5 per 1000 person-life years for non-optimal and optimal responders respectively. In optimal responders, compared with non-optimal responders, the hazard ratio (95% CI) for incident CVD was 0.87 (0.84 – 0.89; $p < 0.0001$). The association was not confounded by family history and other CVD risk factors.

Conclusions: Optimal response to statins within 24 months after initiation was associated with a decreased risk of CVD in UK general population. Further pharmacogenetic studies may indicate reasons for non-optimal response.

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P05.63C

Rare copy number variants involving cardiac function and development genes detected in earthquake induced takotsubo cardiomyopathy patients

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Biological mechanisms underlying stress cardiomyopathy (SCM, also known as Takotsubo cardiomyopathy), are poorly understood. SCM can occur sporadically, often in association with a stressful event, or in clusters after major natural disasters. Our primary study cohort consisted of 28 women who suffered SCM as a result of two devastating earthquakes that struck the city of Christchurch, New Zealand, in 2010 and 2011. Since then, a further 5 cases arising from the Kaikoura 2016 event have also been added to the CNV analysis cohort, bringing the total case number to 33. To seek possible underlying genetic factors, array comparative genomic hybridisation was carried out on these subjects. The most striking finding from these analyses was the observation of a high rate of rare, heterogeneous copy number variants (CNV) of uncertain clinical significance (in 13/33 subjects). Several of these CNVs clearly impacted on genes of cardiac relevance including *RBFOX1*, *GPC5*, *KCNRG*, *CHODL*, and *GPBP1L1*. There is no physical overlap between the CNVs, and the genes they impact do not fall into a clear pathophysiological pathway. However, the recognition that SCM cases display a high rate of unusual CNV, and that SCM predisposition may therefore be associated with these CNVs, offers a novel perspective and a new approach by which to understand this problematic and enigmatic condition.

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P05.64D

Should the minor genes be included in NGS panels for inherited cardiac diseases?

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Introduction: Genetic testing in inherited cardiac diseases has been revolutionized by NGS technology: the NGS-based panel approach, indeed, has enabled to test a large number of genes simultaneously, differently from the traditional Sanger, limited to the most prevalent and characterized genes. Therefore, recently, so called "minor genes" have been included in diagnostic NGS panels, causing interpretative issue, related to the high prevalence of variants of unknown significance (VUS), not clearly associated to the disease. Here, we report our experience with expanded gene panels for genetic testing.

Materials and Methods: 82 consecutive patients, 19 suspected Hypertrophic Cardiomyopathy (HCM), 29 Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), 12 Long QT syndrome (LQTS), 16 Brugada syndrome (BrS) and 6 Idiopathic Ventricular Fibrillation (IVF) patients have been analyzed using 3 different custom sequencing panels, including 11 genes for HCM, 9 for ARVC, 12 for LQTS and IVF and 4 for BrS.

Results: 28 probands carried at least one potentially pathogenic variant (34%). According to the ACMG guidelines, the majority of the identified variants (70%) were VUS, while 18% were Likely Pathogenic (LP) and 12% Pathogenic (P). 30% of the VUS have been identified in minor genes, 8 in the LQTS/IVS and 1 in the HCM panel, respectively. On the other hand, no LP or P variant was identified in minor genes.

Conclusions: These data showed that the inclusion of minor genes in the genetic screening does not significantly increase the detection rate, while it is associated with an increase in the rate of VUS.

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Sudden cardiac death caused by bi-allelic variants in the *PPA2* gene

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Sudden cardiac death (SCD) is one of the commonest causes of mortality in infants and older individuals. Many cases are a result of pathogenic variants in cardiac ion-channel and cardiomyopathy-related genes; however numerous cases remain unexplained. Kennedy *et al*. (2016) and Guimier *et al*. (2016) (both Am J Hum Genet) reported cases of both infantile and alcohol-induced SCD caused by bi-allelic variants in the *PPA2* gene, which encodes a mitochondrial pyrophosphatase essential for cell phosphate metabolism. Here, we report further cases of infantile and possible alcohol-induced SCD caused by compound heterozygosity for *PPA2* variants. Family 1 involved the sudden unexplained death of two infants aged less than 1 year. Post-mortem revealed a structurally normal heart in both. Exome sequencing detected two likely pathogenic *PPA2* variants in both: p.(Ser61Phe) (previously reported by Guimier *et al*.) and p.(Arg127Leu) (previously reported by Kennedy *et al*.). Bi-parental inheritance was confirmed, and we were able to provide predictive genetic testing for a third healthy infant.Family 2 involved the SCD of a 14-year old whose heart showed fibrosis at post-mortem. Two variants were detected in *PPA2* by Sanger sequencing: a likely pathogenic variant, p.(Glu172Lys) (previously detected in a family where acute sensitivity to alcohol manifested as myocardial fibrosis (Kennedy *et al*.) and a VUS, p.(Thr159Met) (not previously reported). Functional studies of p.(Thr159Met) are on-going. These findings add to the previous reports of autosomal recessive SCD caused by pathogenic variants in *PPA2*, and expand the diagnostic pathways available to investigate SCD.

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P05.66B

Missense variants in genes connected with cardiovascular diseases in supercentenarians

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Introduction: During the last decades, mankind suffers unprecedented demographic changes as a result of decreased/controlled birthrates. Cardiovascular,

neurodegenerative and malignant diseases are mostly responsible for populations' mortality. Twin studies show genetic factors play crucial role in longevity. Supercentenarians are individuals who have reached 110 or more years. The analysis of their genomes could help to clarify the role of genetic variants or reclassify others.

Materials and Methods: In publicly available database of supercentenarians (Gierman HJ et al., 2014) are listed altogether 110,000 variants. We selected 216 genes connected with atherosclerotic vessel changes, lipoprotein signaling or cholesterol metabolism and analyzed the nononsynonymous variants according to their clinical significance (ClinVar).

Results: 151 nonsynonymous variants in 216 cardiovascular genes are found in the list of supercentenarians. After applying filtering criteria, we listed 35 variants in 21 genes divided into three categories („Pathogenic„Conflicting interpretation of pathogenicity", and„Protective"). 8 variants are in APOB gene, followed by 3 variants in APOE gene. 16 of 35 variants have MAF<0,01%. Of them only rs769452 in APOE gene is absent in supercentenarians, while seven others are absent in control group.

Conclusion: We particularly focus on variants with unknown significance in supercentenarians. On the basis of our results we could speculate the pathogenic nature of rs769452 and possible protective role of the other seven variants in this study. At the moment we are undertaking "*in silico*" modeling in attempt to reclassify those as "pathogenic" or "benign". Thus it would be easier to make clinical decisions in the future.

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P05.67C

Trans-ethnic genome-wide association studies on time to rejection and death in heart transplant donors and recipients

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Introduction: Heart transplantation is the best therapeutic option for selected patients with end-stage heart failure. However, the immunological barrier between the donor and recipient still limits long-term survival. Immunosuppressive drugs are needed to avoid rejection, but cause an increased incidence of cancer and infections. Besides HLA, also other genetic factors play a role in graft rejection. We aim to identify genetic variants in the patient and in the donor that are involved in rejection and survival after heart transplantation.

Methods: The iGeneTRAiN consortium consists of over 30,000 solid organ transplant recipients and donors. We included 1,043 heart transplant recipients and 783 donors from five different hospitals. We tested over 8 million high quality variants for association with time to rejection, and time to death. We used a mixed models approach to take relatedness and ancestry into account.

Results: We identified nine loci that were significantly associated with rejection ($P < 5x10^{-8}$); two in donors and seven in recipients. In addition, we identified one locus in recipients that was associated with survival.

Conclusion: We identified a total of ten loci that are associated with rejection and survival. We aim to increase our sample of heart transplant donors and recipients in the near future. In addition, we will conduct cross-organ meta-analyses including lung, liver, and kidney transplants, maximizing statistical power to identify novel variants. We ultimately aim to translate genetic data into clinical applications such as more optimal genomic compatibility matching of donor-recipient pairs and immune suppression therapy dosing.

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P05.68D

Whole exome sequencing in 186 sporadic transposition of the great arteries cases reveals complex genetic etiology

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Introduction: Transposition of the great arteries (TGA) is a rare life-threatening congenital heart disease with little known etiology. Some family-based genetic variants are seldom observed in sporadic TGA subjects, indicating a distinct genetic etiology in sporadic TGA. We sought to

explore the genetic etiology of sporadic TGA at different levels including mutations, genes and pathways.

Materials and Methods: 186 sporadic TGA cases and 182 obesity patients without cardiac disease phenotype were submitted to whole exome sequencing. Variants calling and filtering were performed according to GATK best practice pipeline. Single-variant association tests and gene-based burden testing were performed to identify rare, disruptive mutations. We also aggregated mutations in 4434 gene sets and performed gene-set based burden tests.

Results: Few single mutations and genes could achieve exome-wide significance after multiple testing corrections. However, 45 gene sets were found statistically significant by gene-set based burden tests (adjust P-value <0.05). These gene sets were mainly involved in embryonic organ development, cilium organization and cellular response to stimulus, which is consistent with our current understanding about the genetic basis underlying CHD. For example, there was a significant enrichment of rare disruptive mutations in TGA (5.95 mutations per individual) compared to control cohort (4.88 mutations per individual) in the gene set GO_CILIUM_ORGANIZATION (adjust P-value- = 4.36E-06).

Conclusions: Our study revealed a polygenic burden of rare disruptive mutations in sporadic TGA, implying an extensive and complex genetic etiology underlying TGA. The findings of this study may help stratify patients for guiding the therapeutic management of their clinical care.

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P05.69A

IGF1 gene is associated with triglyceride levels in subjects with family history of hypertension from the SAPPHIRe and TWB projects

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Introduction: Chromosome 12q23-q24 has been linked to triglyceride levels by linkage studies, and it contains the *Insulin-like growth factor 1* (*IGF1*) gene. However, association between *IGF1* and triglyceride levels was not well investigated and remained unclear.

Materials and Methods: We investigated the association between *IGF1* and triglyceride levels by using two independent samples collected in Taiwan: the first sample consists of 954 siblings in 397 families from the Stanford Asian Pacific Program in Hypertension and Insulin Resistance (SAPPHIRe); the second sample consists of 13,193 unrelated subjects from the Taiwan biobank (TWB) project. Five tag single-nucleotide polymorphisms (tag-SNPs) within *IGF1* were analyzed.

Results: First, based on the SAPPHIRe sample, we found that one *IGF1* tag-SNP was associated with triglyceride levels (β = -0.049, p = 0.0043). Then, subset analyses in the TWB sample showed that this association appeared in subjects with a family history (FH) of hypertension (β = -0.045, p = 0.0000034), but not in subjects without an FH (p = 0.61). A re-examination of the SAPPHIRe sample confirmed that this association appeared in subjects with an FH of hypertension (β = -0.068, p = 0.0025), but not in subjects without an FH (p = 0.32).

Conclusions: The successful replication in two independent samples indicated that *IGF1* is associated with triglyceride levels in subjects with an FH of hypertension in Taiwan.

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P05.70B

Exome sequencing in disclosing causes of unexpected death in child - single genetic center experience

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Introduction: In the period of last two years in genetic outpatient clinic in Serbia we had six families with a family history of unexpected death of a child.

Methods: We performed exome sequencing for all.

Results: In two cases, there was polylethality with sudden cardiovascular death in a previously healthy child and other family members. We found causative *RYR2* gene variant in first and two variants of unknown significance in *KCNA5* and *FBN1* gene in second family. Pathogenic variant in *ABCD1* gene (X-linked adrenoleucodystrophy) explains fulminant and unexpected death of boy during gastrointestinal infection. *MECP2* gene duplication (associated with infection susceptibility) was discovered by NGS in the boy with intellectual disabilities and epilepsy who died unexpectedly from pneumonia. The girl with undiagnosed metabolic disease without signs of respiratory failure died in sleep; in her sibling we found the pathogenic variants in the *SURF1* gene for COX IV deficiency. The boy with dysmorphic features, epilepsy and sudden death was diagnosed with hemizigot variant in the *HUWE1* gene.

Conclusion: Many genetic diseases, not only cardiovascular, increase risk for unexpected death during childhood. Early genetic referral, genomic testing and genetic counselig are crucial.

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P05.71C

Precision therapy for venous malformations: efficacy and safety of sirolimus in vascular malformations, preliminary results of a phase III clinical trial VASE

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Introduction: Venous malformations (VMs) are caused by activating, somatic TIE2 or PIK3CA mutations, activating AKT and mTOR. In a pilot study, we demonstrated efficacy of sirolimus as a personalised therapy. We subsequently set up a trial to assess the efficacy and safety of sirolimus in a larger cohort of patients (Vascular Anomaly - Sirolimus - Europe, VASE).

Methods: VASE is a prospective, multicentric European phase III cross over clinical trial. 250 patients with various vascular malformations refractory to standard treatments are enrolled. Evaluation includes clinical history and examination, QOL questionnaire, coagulation analysis, and MRI before and after one year of treatment. Genotyping of tissue biopsies is performed.

Results: During 2016-17, 44 patients (median age 44y; 2y-71y), including 31 VMs, 4 lymphatic malformations, 5 capillary-venous malformations, and 4 syndromic patients, were enrolled. Sirolimus was well tolerated with mostly mild and easily manageable side effects. There was no complete response, but 89% of patients (n = 39) presented a rapid clinical improvement with reduction of pain and/or coagulation abnormalities, decrease in size of lesions, and/or improvement in quality of life (QOL). Currently, 29 patients have been treated with sirolimus for ≥12 months and 8 for ≥6 months. The 1-year radiological evaluation demonstrated VM reduction ≥10% in 45% of 21 evaluable patients.

Conclusion: Sirolimus showed impressive efficacy in slow-flow vascular malformations with activation of the

PI3K-AKT-mTOR pathway due to somatic mutations. It reduced pain and improved functional restraint in the majority of patients. This underscores the results of our earlier pilot study on 20 VMs.

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P05.72D

Mutation spectrum in a Kazakhstani cohort with ventricular tachycardia: targeted sequencing study

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Introduction: Ventricular tachycardia (VT) is a common symptom in cardiac disorders of different etiology. **Aim:** to investigate genetic basis of VT in patients with cardiomyopathy in Kazakhstan using targeted NGS (design of new HaloPlex gene panel).

Material and Methods: We enrolled 92 patients, diagnosed with either coronary heart disease (CHD), dilated cardiomyopathy (DCM) or idiopathic ventricular tachycardia (iVT) in a study to evaluate the genetic profile and variants in known 96 cardiac risk genes by targeted next generation sequencing (NGS).

Results: By sequencing 92 clinically well diagnosed patients we observed a total of 168 mutations (61 distinct) listed in the Human Genome Mutation Database (HGMD) and another 256 rare/unique variants with elevated pathogenic potential. The majority of CHD patients carried known mutations and rare variants with high pathogenetic potential in the same genes and at a comparable frequency as observed for DCM and iVT patients. The most abundant mutations observed for the CHD group locate to MYBPC3, DMD, LAMA2, MYH6 and GAA. Mutations in the PRKAG2 gene were overrepresented in the CHD subgroup, correlating to the prominent role of disturbed energy metabolism in CHD development and progression.

Conclusions: Our study indicates that individuals presenting with VT secondary to CHD, DCM or of idiopathic etiology carry multiple rare mutations and potentially pathogenetic sequence variants in classical cardiac risk genes in a similar pattern and at a comparable frequency. This study was supported by a grant from the Ministry Education and Science, Republic of Kazakhstan (AP05134683).

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P05.73A

Deletion of a small fragment of chromosome 7q11.23 containing ELN gene may be responsible for supravalvular aortic stenosis with pulmonary stenosis and no other features of Williams syndrome

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Williams syndrome (Williams Beuren syndrome, MIM 194050) is a multisystem disorder with variable phenotypic expression, caused by microdeletions of chromosomal region 7q11.23. Cardinal features of the syndrome are: supravalvular aortic stenosis (SVAS), mental retardation, and distinctive facial features, commonly known as 'elfin facies'. Size of the deletion may vary, mostly between 1.5 to 1.8 Mbp. Vast majority of cases contains deletion of multiple genes (>25) along with the ELN gene.

We report a 12 month old boy with congenital SVAS and pulmonary stenosis. No dysmorphic features were observed during several hospitalizations and visits in the genetic clinics. Genitourinary, skeletal, skin and endocrine anomalies were excluded, except of subclinical hypothyroidism. Growth parameters are normal. It is too early to determine neurological development in this patient, however no psychomotor retardation was diagnosed so far.

MLPA testing for 7q11.23 region revealed a deletion in the exon 33 of ELN gene. Microarray (aCGH) analysis showed deletion of approximately 68kbp containing part of ELN gene and LIMK1 gene, that is adjacent to ELN.

Isolated SVAS is described as a separate nosological entity in OMIM (#185500). Part of the patients with this diagnosis carry a heterozygous point mutation, or intragenic deletions in ELN gene.

68kb deletion in the 7q11.23 is much smaller than all the other deletions reported in ISCA and Decipher database. This report support the hypothesis that deletion in the

Williams syndrome region limited to ELN gene may cause nonsyndromic SVAS and pulmonary stenosis.

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P05.74B

ZECARDIO: A zebrafish genetic screening platform for cardiovascular disease association studies

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Cardiovascular disease represents a heavy burden for societies and was responsible of the premature death of 17 million people worldwide in 2015. Dilated cardiomyopathy (DCM) is a common form of heart disease with high mortality. It affects 1 in 250 people, principally young adults and children. DCM is characterized by ventricular dilatation and impaired cardiac function, due to a dramatic decrease in ejection fraction. DCM is also associated with increased risks for cardiac arrhythmia and sudden cardiac death. To achieve better patient stratification and identify new druggable targets, which might allow finding novel therapies, it is crucial to identify genes associated to DCM progression. Different NGS studies have identified genetic variants with a possible association to DCM. However, the use of animal models is required to validate phenotypically their real impact in DCM progression. The genetic and physiologic homology between humans and zebrafish allows performing functional genomic studies with this model organism. Hereby, we have developed an experimental platform that combines fast gene inactivation and robust cardiovascular phenotyping in zebrafish: ZeCardio. ZeCardio combines the advantages of CRISPR/Cas9 for generating F0 mutant larvae with a high mutagenesis efficiency rate (CRISPANTS) and a high-throughput imaging system allowing morphological and functional analysis of cardiovascular phenotypes. Such platform represents a fast and reliable screening method for validating novel DCM associated genes/therapeutic targets and, eventually, for discovering novel therapies to treat this disease. *This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 755988.*

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P06 Metabolic and mitochondrial disorders

P06.01A

Variants of human peptide transporter 2 (PEPT2) and chronic kidney disease in acute intermittent porphyria Spanish carriers

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Introduction: Acute intermittent porphyria (AIP) is a haem biosynthesis disorder, leading to an overproduction of δ-aminolevulinic acid (ALA) and porphobilinogen (PBG). AIP is characterized by acute neurovisceral attacks. Chronic kidney disease (CKD) is a long-term complication, correlated with the frequency of attacks. Urinary ALA and PBG seem to promote renal tubular toxicity. Variants of human peptide transporter 2 (PEPT2), which mediates reabsorption of ALA in renal tubular cells, predict CKD in AIP. Our aim was to analyse PEPT2 genotype and the onset of CKD in Spanish AIP carriers.

Materials and Methods: PEPT2 alleles were determined by sequencing and haplotype analysis of two tag SNPs in exons 13 and 15. Urinary PBG and ALA were measured by chromatography and spectrophotometry. Glomerular filtration rate was estimated with CKD-EPI Creatinine Equation (eGFR).

Results: 50 AIP carriers, 52% symptomatic, were included. Multiple lineal regression analysis showed no association between levels of urinary ALA and PBG and PEPT2 genotype. Carriers of at least one PEPT2*2 allele showed higher eGFR (μ: 92,08 ml/min per 1.73 m2; SE:5.22) compared with PEPT2*1/*1 carriers (μ: 78.37 ml/min per 1.73 m2; SE: 5.54), but with moderate statistical evidence ($p = 0.07$). 28.57% of PEPT2*1/*1 carriers, 16.67% of *1/*2 carriers and 0% of *2/*2 carriers presented

eGFR<60 ($p = 0.21$). The occurrence of acute attacks was associated with lower eGFR and higher prevalence of CKD ($p < 0.01$).

Conclusions: In our population, PEPT2 genotyping may be a useful tool to predict risk to CKD although the occurrence of acute attacks seems to be a more powerful predictor.

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P06.02B

Aberrant regulation of the GSK-3β/NRF2 axis unveils a novel therapy for adrenoleukodystrophy

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NRF2, encoded by *NFE2L2* gene, is the master regulator of endogenous antioxidant responses. Oxidative damage is a shared and early-appearing feature in X-linked adrenoleukodystrophy (X-ALD) patients and the corresponding mouse model (*Abcd1*$^{-}$ mouse). X-ALD is a rare neurometabolic disease caused by the loss of function of the peroxisomal transporter ABCD1. In mice, *Abcd1* loss results in a phenotype similar to adrenomyeloneuropathy (AMN), the most common X-ALD phenotype. Here, we identify an impaired NRF2 antioxidant response caused by aberrant activity of GSK-3β. We find that GSK-3β inhibitors can significantly reactivate the blunted NRF2 response in patients' fibroblasts. In the mouse models (*Abcd1*$^{-}$ and *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice), oral administration of dimethyl fumarate (DMF/ BG12/Tecfidera), an FDA-approved NRF2 activator, normalized i) mitochondrial depletion, ii) bioenergetic failure, iii) oxidative damage and iv) inflammation, highlighting an intricate cross-talk governing energetic and redox homeostasis in X-ALD. Strikingly, DMF halted axonal degeneration and locomotor disability. Our results indicate that therapies activating NRF2 hold therapeutic potential for X-ALD and other axonopathies with impaired GSK-3β/NRF2 axis. Supported by grants from the Spanish Institute for Health Carlos III and 'Fondo Europeo de Desarrollo Regional (FEDER), Union Europea, una manera de hacer Europa' [PFIS FI12/00457] to P.R-R., FIS PI14/00410 to A.P., FIS PI15/00857 to S.F.

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P06.03C

26 novel homogentisate 1,2, dioxygenase (*HGD*) gene variants, *in vitro* splicing analysis and genotype-phenotype correlations in the largest cohort of patients with alkaptonuria (AKU)

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We present the systematic analysis of the largest cohort of patients with the rare metabolic disorder alkaptonuria (AKU). In 166 patients, including those from the SONIA2 (Suitability of Nitisinone in Alkaptonuria 2) and SOFIA (Subclinical Ochronotic Features In Alkaptonuria) studies, we identified 22 novel homogentisate 1,2-dioxygenase (*HGD*) gene variants by DNA sequencing, and four novel larger genomic deletions within *HGD* gene by MLPA (Multiplex Ligation-dependent Probe Amplification). In addition, we analyzed by minigene reporter assay seven novel or previously reported *HGD* variants predicted to affect splicing. Two of them were shown to cause exon skipping or cryptic splice-site activation. Thus, using DNA sequencing, MLPA and in *vitro* splicing analysis we were able to identify AKU-causing mutation in 328 of 332 AKU chromosomes (98.8%). However, in two patients, only one *HGD* mutation was found, and in one case, no *HGD* variant has been identified. No patient's cDNA is available in order to verify whether some deep intronic *HGD* variants affecting correct exon splicing represent an alternative mutation mechanism in these cases. For the first time in AKU, we performed a genotype-phenotype correlation study for the four most frequent *HGD* mutations (G161R, M368V, A122V, ivs1-1G>A), identified in 139 patients participating in the SONIA2 study. We found no correlation of either urine excretion or serum HGA concentrations to a specific *HGD* genotype. Most probably, these parameters are not suitable for this kind of analysis, since they are affected by external factors, such as renal function and dietary intake of tyrosine. (7FP- DevelopAKUre 304985)

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P06.04D

Farber disease (acid ceramidase deficiency): time to diagnosis is influenced by disease severity, indicating that more slowly progressive disease may be underdiagnosed

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Introduction: Farber disease is an ultra-rare autosomal recessive lysosomal storage disease characterized by deficiency of the enzyme acid ceramidase, caused by mutations in the *ASAH1* gene. Resulting accumulation of the pro-inflammatory and pro-apoptotic sphingolipid ceramide causes typical symptoms with a broad spectrum of severity.

Materials and Methods: A review of 96 case studies of Farber patients revealed 64 patient cases where time to diagnosis could be estimated.

Results: Diagnosis is generally based on the triad of (1) hoarse or weak voice, (2) joint inflammation and contractures, and (3) subcutaneous nodules. Patients with a more severe, rapidly progressive, phenotype often manifest with the triad of symptoms in early infancy, and also exhibit signs of central nervous system (CNS) and respiratory involvement. Patients with more moderately progressive phenotypes often present with one of the classic symptoms during early childhood and develop the full triad over months to years. The average and median times to diagnosis (TTD) for severe phenotype patients was significantly shorter than the average and median TTDs for moderate phenotype patients. Generally, the emergence of all three typical symptoms led to the suspicion of Farber disease and genetic and/or enzyme activity testing.

Conclusions: The analysis indicates that TTD is longer in those patients with more moderate and attenuated phenotypes, potentially due to the low index of suspicion in patients who may have not yet developed all three typical symptoms, and points to the need for additional education on the broad phenotype spectrum and indications for testing of acid ceramidase deficiency.

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P06.05A

The first manifesting case of Barth syndrome in a heterozygous female patient with normal karyotype

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Introduction: Barth syndrome (BTHS) is an X-linked recessive disease caused by mutations in tafazzin gene (TAZ) which lead to cardiolipin deficiency and mitochondrial dysfunction. Male patients have variable clinical findings - cardiomyopathy, skeletal myopathy, prepubertal short stature, neutropenia, 3-methylglutaconic aciduria. Female carriers are usually asymptomatic.

Materials and Methods: We report male and female siblings with left ventricular noncompaction and hypotonia. At age of three months, female was found to have mild aortic valve stenosis with increased trabeculations of the left ventricle. At age 9 years she had noted a mild persistent muscle weakness and easy fatigability. The patient had a normal female karyotype. No biochemical abnormalities.

Her younger brother showed a severe phenotype of BTHS - dilated cardiomyopathy with noncompaction of the left ventricle and endocardial fibroelastosis, cyclic neutropenia, muscle weakness. Metabolic testing showed elevated levels of urine 3-methylgultaconic acid and 3-methylglutaric acid.

Results: The molecular genetic testing showed that both siblings carry a novel mutation: c.253insC, p. (Arg85Profs*54) in exon 3 of the TAZ gene. The heterozygous female patient is manifesting carrier while the severely affected male proband was hemizygous for the X-linked TAZ gene mutation.

Conclusions: We identified a novel TAZ gene insertion that is associated with a classical phenotype of BTHS in the male sibling and a milder clinical presentation in his sister. This is the first report of a manifesting female carrier of BTHS.

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P06.06B

Clinical, biochemical and genetic spectrum of 70 patients with ACAD9 deficiency: is riboflavin supplementation effective?

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Mitochondrial acyl-CoA dehydrogenase family member 9 (ACAD9) is an essential assembly factor of mitochondrial respiratory chain complex I. The clinical presentation of ACAD9 deficiency is dominated by cardiomyopathy. Other features are lactic acidosis, myopathy and developmental delay. Here we describe the genetic, clinical and biochemical findings in a cohort of 70 patients, of whom 29 previously unpublished. Among the disease-causing biallelic *ACAD9* variants identified, 34 were known and 18 previously unreported variants. No patients harbored biallelic loss of function mutations, indicating that this combination is unlikely to be compatible with life. For the causal pathogenic variants distributed across the gene, no obvious genotype-phenotype correlation was observed. The majority of the patients presented in the first year of life. For this subgroup the survival was poor (50% not surviving the first two years) comparing to patients with a later presentation (more than 90% surviving 10 years). The most common clinical findings were cardiomyopathy (85%), muscular weakness (75%) and exercise intolerance (72%). Interestingly, severe intellectual deficits were only reported in one patient and severe developmental delays in just four patients. The majority of the patients (70%) were able to perform normal daily-life activities. Remarkably, our results show that riboflavin treatment improves complex I activity for most of patient-derived fibroblasts tested. This effect was also reported for the majority of the treated patients and is mirrored in the survival data. In the patient group with disease-onset below one year of age, a statistically-significant better survival for patients treated with riboflavin was observed.

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P06.07C

The Beginning of the Neonatal Screening for Congenital Adrenal Hyperplasia in Lithuania: Cut-off Limits Based on 27175 Infants

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Introduction: Setting population specific cut-off levels is essential in cost-effective newborn screening programs. This study describes the results obtained in the first year of a public congenital adrenal hyperplasia (CAH) screening program in Lithuania.

Materials and Methods: 17-alpha-hydroxyprogesterone (17-OHP) concentrations in dry blood spots from 27175 neonates were measured using a neonatal screening test. 17-OHP concentrations were compared in accordance to gender, gestation status, 5 gestational age (GA) categories, 6 gestational weight (GW) categories and the age at blood sampling (ABS). 95th percentile cut-off values were calculated for GA and GW categories and evaluated using data of 4 confirmed CAH cases. Analysis was significant at $P < 0.05$.

Results: A total of 27175 infants (13142 females, 13911 males, 122 unknown) were screened. 1469 (5,4%) newborns were born at pre-term (PT) and 25706 (94,6%) at full-term (FT). Mean 17-OHP concentrations were significantly higher in PT vs FT group (28.99 ± 31,68 mmol/l vs 11,9 SD ± 9.0 mmol/l, $P < 0.05$). Mean 17-OHP was higher in males (13.46 ± 12.63 mmol/l vs 12.13 mmol/l, $P < 0.05$). Lower GW and GA categories had higher mean 17-OHP concentrations ($P < 0.05$). No significant correlation was found between ABS and 17-OHP concentration. Finally, all 4 cases would be detected by proposed cut-off values.

Conclusions: Birth at preterm, lower gestational age, lower gestational weight and male gender were associated with higher 17-OHP concentration. The sensitivity and specificity of the cut-off values needs to be evaluated by further studies.

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P06.08D

The frequency of mutations and polymorphisms in the genes involved in the metabolism of copper (ATP7B, COMMD1 and XIAP)

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Wilson disease (WD) is a rare inherited disorder associated with the copper accumulation in different organs. WD is caused by mutations in the *ATP7B* gene. The clinical phenotype of WD is modified by variants in other genes including *COMMD1* and *XIAP*. Protein COMMD1 takes part in the transport of copper. XIAP is antiapoptotic protein, whose activity depends on the cytoplasmic copper concentration. Hereby the interactions of these proteins can be involved in a clinical polymorphism of the WD.

Materials and Methods: We examined 89 WD patients by NGS method. We designed targeted panel NimbleGen SeqCap EZ Choice: 151012_HG38_CysFib_EZ_HX3 (ROCHE) for analysis *ATP7B*, *COMMD1* and *XIAP* genes. Different algorithms were used to predict the effects of the mutations.

Results: 34 mutations in *ATP7B* gene were detected. Two most frequent mutations were c.3207C>A (48% of alleles) and c.3190G>A (7% of alleles). Single rare mutations were found in 29% of cases. All our patients had mutations of both copies of the *ATP7B*. Two patients were heterozygous carriers of variants in *COMMD1* (c.180+4C>T, c.340C>T). Two patients had variants c.925G>C, c.355A>G in *XIAP* gene; benign variant c.1268A>C were detected in our group very often (63% of cases).

Conclusion: We discovered no significant pathogenic mutations in the *COMMD1* gene in patients but found two polymorphisms. We have found 3 polymorphisms in the *XIAP*. The meaning of these results remains unclear. The study was implemented under the Russian Science Foundation grant №14-50-00069.

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P06.09A

Increased bile acid synthesis and near-absence of gut-derived hormones in a patient with neonatal diabetes and severe diarrhea caused by mutations in the neurogenin-3 gene

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Introduction: A Chilean proband diagnosed with permanent neonatal diabetes and severe malabsorptive diarrhea was found to carry two loss-of-function mutations in the NEUROG3 gene (c.82G>T and 404T> C).

Aim: to measure gut-derived hormones during a meal test to gain insights on pathophysiological processes that may explain the impaired intestinal function of the proband carrying NEUROG3 mutations.

Subjects and Methods: Proband, parents and controls were submitted to a standardized Oral Liquid Meal Test (237 ml; 220 Kcal; 29 g CHO) with measures of plasma levels of Gastric Inhibitory Polypeptide (GIP), Glucagon-Like Peptide-1 (GLP-1), Pancreatic Polypeptide (PP), Fibroblast-Growth-Factor-19 (FGF-19), C-peptide, 7α-hydroxy-4-cholesten-one (C4) and platelet serotonin.

Results and Discussion: Near-absence of GIP, GLP1, PP and C-peptide were found during the meal test in the proband, in contrast to controls. FGF19 plasma levels were extremely low in the proband (26.3 pg/ml versus 118 ± 63 pg/ml in $n = 7$ controls), while C4 levels were very high (83.4 ng/ml vs 16.3 ± 12 ng/ml in $n = 47$ controls). Interestingly, platelet serotonin levels, which reflects its gut-derived production, were extremely low in the proband (27 ng/109 platelets vs 772 ± 239 in $n = 64$ controls). Values of plasma C4 and platelet 5HT of the patient felt outside the prediction limits for univariate normal distribution.

Conclusion: The proband with loss-of-function mutations in NEUROG3 gene displays extremely-low platelet serotonin levels and plasma concentrations of gut-derived incretins. The combination of low plasma FGF19 and very high C4 plasma levels suggests an increased hepatic synthesis of bile acids as a contributor of the severe malabsorptive diarrhea. FONDECYT 1150416.

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P06.11C

Plasma and urinary metabolomic profiles of Down syndrome correlate with alteration of mitochondrial metabolism

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Introduction: Down syndrome (DS) is caused by the presence of a supernumerary copy of the human chromosome 21 (Hsa21) and is the most frequent genetic cause of intellectual disability (ID). Key traits of DS are the distinctive facies and cognitive impairment.

Materials and Methods: We conducted for the first time an analysis of the Nuclear Magnetic Resonance (NMR)-detectable part of the metabolome in plasma and urine samples, studying 67 subjects with DS and 29 normal subjects as controls selected among DS siblings.

Results: Multivariate analysis of the NMR metabolomic profiles showed a clear discrimination (up to of 80% accuracy) between the DS and the control groups. The univariate analysis of plasma and urine revealed a significant alteration for some interesting metabolites. Remarkably, most of the altered concentrations were consistent with the 3:2 gene dosage model, suggesting effects caused by the presence of three copies of Hsa21 rather than two: DS/normal ratio in plasma was 1.23 (pyruvate), 1.39 (fumarate), 1.47 (succinate), 1.33 (lactate), 1.4 (formate).

Conclusions: Several significantly altered metabolites are produced at the beginning or during the Krebs cycle. Accounting for sex, age and fasting state did not

significantly affect the main result of both multivariate and univariate analysis.

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P06.13A

Hematopoietic stem cell transplant does not prevent neurological deterioration in infants with Farber disease

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Farber disease (FD) is an inherited autosomal recessive disorder of lipid metabolism. The hallmark of the disease is systemic accumulation of ceramide due to lysosomal acid ceramidase (ACDase) deficiency. The involvement of the central nervous system is critical in this disorder leading to rapid deterioration and death within a few years after birth. Efforts to treat patients by hematopoietic stem cell transplant (HSCT) have resulted in favorable results in the absence of neurological manifestations. We report the outcomes of HSCT in two patients with FD who received early HSCT and had neurological deterioration post-transplant. We also present a new understanding of the limitations of HSCT in FD management based on our observations of the clinical course of the two patients after therapy. <!--End-Fragment-->

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P06.14B

Novel *FDXR* pathogenic variants expand the clinical spectrum related to human ferredoxin reductase defects

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Introduction: *FDRX* gene encodes the mitochondrial membrane-associated ferredoxin reductase, the sole enzyme essential for the biosynthesis of iron-sulfur (Fe-S) clusters and heme formation. Fe-S clusters proteins are involved in enzymatic catalysis and gene expression, mitochondrial respiration, DNA replication, DNA repair, and iron homeostasis. Recently, *FDXR* defects have been shown to cause a novel mitochondrial disease with auditory neuropathy and optic atrophy.

Patients and Results: Here, we describe nine novel *FDXR* pathogenic variants revealed by whole exome sequencing of 8 affected individuals from 6 families. The clinical presentation in these individuals is varied, and includes families with more severe phenotypes then previously described. Early-onset progressive leukoencephalopathy and brain atrophy, Leigh syndrome, and medullary and cerebellar atrophy are reported in three families. Visual and hearing impairment, in keeping with the previously described phenotype in *FDXR* defects, is reported in four of five investigated families.

Functional tests performed in two available fibroblast cell lines confirm reduction in the FDXR protein level on Western blot.

Conclusions: Our study contribute to the further delineation of molecular and clinical spectrum related to *FDRX* defects.

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P06.15C

Prenatal exome sequencing identifies compound heterozygous *GLB1*-mutations in a fetus with ultrasound abnormalities

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Introduction: The sensitivity and specificity of prenatal ultrasound diagnostics significantly improved over the last years and fetal anomalies are currently being detected in up to 3% of pregnancies. Exome sequencing is underway to become a routine assay in the downstream diagnostic algorithm. However, the clinical interpretation of identified variants remains challenging due to limited and mostly non-standardized prenatal phenotypic features for most disease-associated genes

We report on a fetus with multiple ultrasound abnormalities including increased nuchal translucency, ascites, hydrops fetalis, and left-sided hydrothorax. Thoracentesis of the fetus was performed to relieve the fetal lung and pleural effusion was used for genetic testing. Cytogenetic analysis showed a normal female karyotype. In addition, a fetal blood sample was used to exclude spherocytosis. The fetus had a highly increased number of reticulocytes.

Results: Prenatal exome sequencing identified compound heterozygous mutations in *GLB1*, a previously reported pathogenic missense variant on the paternal allele and a novel nonsense variant on the maternal allele. Recessive-type *GLB1 variants have been associated with the* lysosomal storage disorder GM1 gangliosidosis which is characterized by progressive neurodegeneration.

Conclusion: After the exclusion of aneuploidy, exome-based trio analysis is a fast and cost-efficient diagnostic tool in cases with multiple unspecific fetal anomalies. On the one hand establishing a diagnosis enables an informed choice about future pregnancies and provides information on the prognosis and the recurrence risk, on the other hand unclear results may cause uncertainty. Thus the challenge remains to offer trio-exome analysis in a responsible manner in prenatal diagnostic settings.

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P06.16D

Unusual diagnosis of inflammatory bowel disease in a patient with type 1 Gaucher disease

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Gaucher disease [GD] is an autosomal recessive lysosomal storage disease caused by mutations in the *GBA* gene resulting in deficient glucocerebrosidase activity and accumulation of glucosylceramide [GL1] in reticuloendothelial cells. The pathophysiologic mechanism of GD is multifactorial including cell engorgement, as well as a lipid-specific immune response to accumulating GL1 and lyso-GL1. The non-neuronopathic form, type 1, is more common in the Ashkenazi Jewish [AJ] population with an estimated prevalence of ~1:850. We describe a 16 year old male with paternal AJ ancestry who presented at age 3 with splenomegaly. Reduced glucocerebrosidase activity and compound heterozygosity (N370S/L444P) in the *GBA* gene were consistent with a diagnosis of type 1 GD [GD1]. At age 12 the patient was noted to have anemia, hematochezia, weight loss and diarrhea; he was diagnosed with inflammatory bowel disease (IBD). His father and paternal aunt also have IBD. This umbrella term refers to a state of chronic inflammation of the gastrointestinal tract with an autoimmune etiology. The prevalence of IBD in the Western world is >0.3% with a higher prevalence in the AJ population. Surprisingly, despite the higher prevalence of both GD and IBD in the AJ population, to our knowledge no case of co-occurrence of these two diagnoses has been reported. This suggests the possibility that the GD1 inflammatory state may have an effect on the expression of IBD. Relevant registry data will be reviewed and described and treatment implications will be discussed.

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P06.18B

Glycogen storage disease type III: identification of the first case of uniparental disomy and two novel deletions

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Introduction: Glycogen storage disease type III (GSDIII) is caused by mutations of *AGL* gene with debranching enzyme deficiency.

Materials and Methods: Molecular analysis of three GSDIII patients was performed through Sanger and Next Generation Sequencing; CGHarray e SNParray were used for further genetic investigations. Besides typical clinical features of GSDIII, patient 1 showed a severe growth retardation (<3 SD), whereby endocrinological studies resulted negative.

Results: Patient 1 showed the homozygous variant c.3904insA, patient 2 resulted in apparent homozygosity for W1401X mutation, patient 3 carried a homozygous deletion of the first 4 exons of *AGL*. Since discordant results from segregation studies showed the carrier status in only one parent of patients 1 and 2, further investigations revealed a paternal disomy of chromosome 1 (UPD1) in patient 1, and a paternal inherited 349 kb deletion of chromosome 1 including *AGL* gene in patient 2.

Conclusions: The genetic characterization of three GSDIII patients allowed to describe the first case of GSDIII resulting from UPD1 and two new deletions of the *AGL* gene. Moreover, UPD can play an important role even in case of imprinted genes. Particularly, *ARHI* is a maternally imprinted tumor suppressor gene, implicated in growth and oncogenesis. It could be speculated that *ARHI* overexpression could have a role in the severe short stature of patient 1 with paternal UPD1. The study emphasizes the importance of parental segregation studies especially in patients with recessive conditions to look for specific genetic causes of disease and to estimate properly the risk of family recurrence.

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P06.19C

Clinical and Genetic Analysis in a Rare Case of Hereditary FructoseIntolerance

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Hereditary fructose intolerance is a very rare autosomal recessive, metabolic disorder with unknown global prevalence. Carrier frequency is estimated at 1 in 70 individuals, especially in those of Caucasian origin. The key enzyme in fructose metabolism is aldolase B produced by the liver at low constant levels; when dietary fructose is ingested, the enzyme becomes active and metabolizes the fructose-1-phosphate and 1,6-biphosphate to 3-phosphate-glyceraldehyde. Due to mutations in the *ALDOB* gene (located on chromosome 9q31.1), the enzyme activity can be drastically reduced (85-100%). In Europe, more than 80% of mutations are A150P, A175D and N335K. Here, we present a case of a 15 months year old patient with a history of liver insufficiency, currently with hepatomegaly, failure to thrive, fever and diarrhea. In evolution, the patient became comatose (Glasgow score 8) with severe hypoglycemia but without ketonuria. Clinical and paraclinical testing raised the question of hereditary fructose intolerance *vs.* hydroxyglutaric aciduria. Genetic testing revealed that the patient was a compound heterozygote with A174D (c.524C>A) mutation in exon 5 and 4 bp deletion in exon 3 (c.113-1_115) of *ALDOB* gene. Subsequent genetic testing of parents showed that they were both heterozygotes: the mother with A174D (c.524C>A) mutation and the father with 4bp deletion in exon 3. The particularities of this case are the presence of liver insufficiency and coma in a child, without ketonuria and neurological long-term consequences, that was eventually diagnosed as a rare case hereditary fructose intolerance.

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P06.20D

Clinical Manifestations and Molecular Aspects of Phosphoribosylpyrophosphate Synthetase Superactivity in Females

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Objectives: Phosphoribosylpyrophosphate synthetase (PRPS) superactivity is an X-linked disorder characterized by urate overproduction (OMIM 300661). This condition is

thought to rarely affect women, and when it does, the clinical presentation is mild. We describe a 16 year old African-American female who developed progressive tophi, nephrolithiasis, and acute kidney failure due to urate overproduction. Family history included a mother with tophaceous gout who developed end-stage kidney disease due to nephrolithiasis and an affected sister with polyarticular gout.

Methods: Whole exome sequencing was performed in affected females and their fathers.

Results: Mutational analysis revealed a new c.520G>A (p.G174R) mutation in the *PRPS1* gene. The mutation resulted in decreased PRPS inhibition by ADP.

Conclusions: Clinical findings in previously reported females with PRPS superactivity showed a high clinical penetrance of this disorder with a mean serum urate level of 8.5 ± 4.1 mg/dl (506 ± 247 umol/L) and a high prevalence of gout. These findings indicate that all women in families with PRPS superactivity should be genetically screened for a mutation (for clinical management and genetic counseling). In addition, women with tophaceous gout, gout presenting in childhood, or a strong family history of severe gout should be considered for *PRPS1* mutational analysis.

K. Hodaňová: None.

P06.21A

Exploring the possible role of PCBP2 in hereditary hemochromatosis

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Introduction: Over the last decade, many mechanisms orchestrating iron metabolism have been highlighted. A number of molecules involved in iron homeostasis have been progressively identified, shedding light into hereditary conditions leading to iron excess or deficiency. Among them, the recently recognized iron chaperone poly(rC)-binding protein 2 (PCBP2) seems to have a crucial role in transferring intracellular iron to the iron exporter ferroportin1. The aim of this study was to investigate PCBP2 as a candidate gene in disorders of iron metabolism. We analysed PCBP2 as a possible modifier in type 4 hemochromatosis and as a determinant in cases with primary iron overload.

Material and methods: 24 patients, belonging to 8 distinct genealogies with type 4 hemochromatosis and showing both high interfamily and intrafamily variability in iron overload degree were investigated for PCBP2 variants. At the same time, PCBP2 was studied in 30 unrelated individuals with hemochromatosis but not carrying mutations in the HFE, TFR2, HAMP, HJV and SLC40A1genes. The entire coding region and the intron-exon boundaries of PCBP2 were sequenced with Sanger method in these patients.

Results: We did not identify any PCBP2 point variant in the examined sample.

Conclusion: Our study was not able to confirm the hypothesis of PCBP2 as a modifier gene in type 4 hemocromatosis. Also, PCBP2 did not appear to be causative of our cases with non-molecularly characterized hemochromatosis. We believe that the analysis should be expanded to more patients to get conclusive considerations on PCBP2 as a gene possibly involved in iron overload disorders.

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P06.22B

In vivo effect of pyridoxine administration on CBS mutants

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Introduction: Pathophysiology of pyridoxine responsiveness in patients with homocystinuria is elusive with a possible chaperoning activity of pyridoxal 5´-phosphate. However, direct demonstration of *in vivo* effect on mutant enzyme activity is lacking. We described previously that cystathionine-beta-synthase (CBS) is released into circulation from several organs, mostly from liver. Here we used this method to assess *in vivo* effect of pyridoxine administration on the rescue of CBS-mutant in 15 patients with homocystinuria.

Methods: We analyzed plasma CBS activity in 8 pyridoxine non-responders (homozygotes/compound heterozygotes for mutations p.A69fs*94; p.C165Y; p.A71Pfs*24; p.V10Wfs*71; p.K211*; p.G246Dfs*52; p.W409_G453del; p.A155T; p.E144K) and 7 partial/full responders (homozygotes/compound heterozygotes carrying mutations p.P145L; p.I278T; p.P49L; p.R336H; p.R336C on at least one allele). CBS activity was measured by LC-MS/MS.

Results: Plasma CBS activity ranged 0-0.7% of controls in non-responders even on combined therapy with methionine restriction, pyridoxine and/or betaine. In contrast, plasma CBS activity in responders not taking pyridoxine was 5.1% (range 0-20.3%) and significantly increased on pyridoxine to 26.2% (range 7.3-72.1%) indicating *in vivo* rescue of the activity of mutant CBS in liver.

Conclusion: Pyridoxine administration partially rescued *in vivo* enzyme activity of selected CBS-mutant. On the other hand large doses of pyridoxine did not increase the activity of CBS in non-responsive patients. This study supports the recent recommendation, that pyridoxine therapy is not necessary in non-responsive patients with homocystinuria. Supported from institutional projects RVO-VFN64165 and ProgresQ26.

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P06.23C

The genetic origin of Hunter syndrome in Belarus

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Introduction: Mucopolysaccharidosis type II (MPS II; Hunter syndrome; OMIM 309900) is a life –limiting, multisystemic disease with varying presentation and severity. This X-linked lysosomal storage disorder is caused by mutations in IDS gene, encoding the lysosomal enzyme iduronate-2-sulfatase (EC 3.1.6.13). For clinical purposes, patients are generally considered to be in one of two categories according to the presence or absence of cognitive impairment. Here we present the results of IDS gene mutations analysis in Hunter patients from Belarus.

Materials and Methods: From early 1980-th 44 MPS II patients were diagnosed in Belarus giving an estimated incidence of 1.2 per 100,000 live births. Patients belong to several generations of 30 families with female-carriers. The genomic DNA from blood leukocytes of the patients and carriers from 17 families was isolated and the entire coding region and flanking intronic sequences of IDS gene were amplified by PCR. Amplified PCR products were purified and sequenced directly by an ABI 3500 Genetic Analyzer.

Results: 10 different mutations were identified: exon 3 - c.236G>A (p.R88H), exon 5 - c.511T>G (p.C171G), exon 6 - c.788delC (p.Y264Tfs*17), exon 7 - c.1004A>G (p.H335R), exon 8 - c.1007-2delA, c.1035G>T (p.W345C), c.1085_1086delTA (p.Y362Cfx*249), exon 9 - c.1402C>T (p.R468W), c.1403G>A (p.R468Q), c.1425G>A (p.W475X). Only the mutations of exon 9 were revealed in more than one family, all others were unique.

Conclusions: The mutation of exon 5 - c.511T>G is novel, never reported before in patients with Hunter syndrome and predicted to be pathogenic.

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P06.24D

The spectrum of pathological sequence variants in patients with lipid metabolism disorder in the Czech Republic

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Introduction: Disorders of lipid metabolism are very common. Hypercholesterolemia plays an important role in the pathogenesis of atherosclerosis and can be effectively treated by lifestyle changes and drugs. Predominantly, the clinical phenotype is caused by mutations in the *LDLR* or *APOB* genes, but also mutations in other genes rarely result in monogenic hypercholesterolemia.

Materials and Methods: Next generation sequencing of the *LDLR*, *PCSK9* and *APOE* genes and part of *APOB* gene (exon 26) using ADH Master kit (Multiplicom, Belgium); Sanger sequencing of the *ABCG5*, *ABCG8* and *LDLRAP1* genes. DNA samples have been collected within the framework of the MedPed project.

Results: We assessed a spectrum of mutations in more than 3900 unrelated patients with clinical diagnosis of hypercholesterolemia. Predominantly, we have found mutations in the *LDLR* gene (22.3%) and in the *APOB* gene (11.0%). 208 unique allelic variants in the *LDLR* gene have been detected. We have found also pathological variants in the *PCSK9*, *ABCG5* and *LDLRAP1* genes. ACMG criteria were used for pathogenicity evaluation of detected sequence variants.

Conclusions: Our study presents a large spectrum of causative mutations, including mutations in rarely involved genes, in Czech patients with lipid metabolism disorders.

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P06.25A

Two novel mutations in ALPL gene associated with mild phenotype of hypophosphatasia in Russia cohort study

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Introduction: Hypophosphatasia (HPP) is a rare heritable metabolic disorder characterized by defective mineralization of bone and/or teeth in the presence of reduced activity of unfractionated serum alkaline phosphatase (ALP). Late forms of HPP (childhood, adult, odontohypophosphatasia) are predominantly caused by heterozygous and compound-heterozygous missense-mutations in *ALPL* gene and often remain undiagnosed. Genetic analysis provides determining of diagnosis in cases with suspected HPP. Furthermore it expands our knowledge of mutation spectrum in mild and latent forms of the disease.

Materials and Methods: We analyzed genomic DNA samples from 55 unrelated individuals with signs of HPP. Minimal criteria's to include in study were recurrent low levels of ALP and low growth. Primers' system for Sanger sequencing was designed and validated for 2-12 exons of *ALPL* gene. First exon of this gene was excluded because it's non-coding and GC-rich region.

Results: Among cohort of patient we detected 9 pathogenic mutations (16,4% detection rate): seven in heterozygous and two in compound-heterozygous. Most of identified mutations were p.E191K (6 times). Furthermore 2 novel missense mutation were detected - p.N323I in exon 9 (heterozygous) and p. Y101C in exon 5 (compound heterozygous with p.E191K in patient with childhood form of HPP).

Conclusions: We presented the first data of *ALPL* gene mutation spectrum in North-Western region of Russia. Most of identified mutations were heterozygous that confirm dominant-negative effect in late forms of HPP. Two novel missense mutations were detected and associated with mild phenotype of disease. This study was supported by Russian Science Foundation №14-50-00069.

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P06.26B

Mutation of IARS2 causes cataracts, growth hormone deficiency, sensorimotor polyneuropathy, sensorineural hearing loss, short stature, and type II esophageal achalasia: expanding the clinical phenotype

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The gene *IARS2* encodes a mitochondrial isoleucyl-tRNA synthetase, a highly conserved nuclear encoded enzyme required for the synthesis of charged tRNA for translation. Recently, an extended French-Canadian family and a Danish proband have associated biallelic pathogenic variants in *IARS2* with a rare autosomal recessive syndrome abbreviated CAGSSS characterized by **ca**taracts, **g**rowth hormone deficiency, **s**ensorineuropathy, **s**ensorineural hearing loss, and **s**keletal dysplasia. Genomic DNA from an

Iranian proband with distinctly overlapping features to CAGSSS was subjected to whole exome sequencing and bioinformatics analysis. This revealed a novel homozygous missense variant in exon 21 (c.2625C>T, p.Pro909Ser) that was included in a 14.3 Mb run of homozygosity affecting the same proline residue described in the original French-Canadian kindred. This study reveals an expansion of the CAGSSS phenotypic spectrum to include type II esophageal achalasia and proposes the bone phenotypes of this syndrome may also appear as a mild manifestation. Furthermore, patient-derived fibroblasts showed normal respiratory chain enzyme activity, as well as unchanged OXPHOS protein subunits and IARS2 levels. A comparative *in silico* protein structural analysis provides a possible explanation for the comparatively mild phenotype compared to the other reported patients with *IARS2* pathogenic variants. Our findings provide further support that biallelic mutations in IARS2 result in an extremely rare but distinctive and clinically recognisable phenotype in human.

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P06.27C

Rapid whole-exome sequencing (WES) in the critically ill infants hospitalized in the intensive care units

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Among congenital metabolic diseases mitochodrial disorders (MD) are the most common. This highly heterogeneous (genetically and clinically) group of disorders is caused by mutations in the mitchondrial or the nuclear genome. Every organ or tissue could be affected, althought the symptoms from the CNS and skeletal muscles are the most common.

We present four unrelated infants with MD manifested by multi-systemic failures and where ealier metabolic and genetic examinations haven't confirmed the diagnosis, for which Rapid WES was perfomed.

WES was performed using SureSelect V5 kit and HiSeq1500 sequencing. Results were avaible after 5-14 days and a genetically confirmed diagnose was made for every patient (3 in life,1 postmortom). The following disorders were diagnosed: Leigh syndrome, Alpers syndrome, pyruvate carboxylase deficiency and in one patinet with suspicion of MD two heterozygous mutations in *TRMT10C* were identified. The results allowed to estabilish prognosis, therapical decissions (in 3 of them hospis care was taken) and familial councelling.

MDs are severe, progressive and often because of their multi-systemic character lead to early death. Lack of time is the biggest problem for physicians and parents, that's why Rapid WES is a unique diagnostic tool and should be performed in cases where a MD is suspected in critical ill children hospitalized in the intensive care units with rapid progression of severe, life-threatening symptoms, to definite diagnosis on the genetic level.

Onset of symptoms (age)	1 day	3 month	5 month	1 day
Death (age)	1,5 month	10 month	–	6 week
Suspected disorder	Mitochondrial disorder, Van der Knapp or Canavan disease	Leigh-like syndrome	Leigh-like syndrome	Mitochondrial disorder
Performed genetic tests before WES	–	Panel of genes (selected exons) responsible for Leigh and Leigh-like syndromes		–
Time for WES results	14 days	7 days 7 days		5 days
Gene	*PC*	*POLG1*	*SCO2*	*TRMT10C*
Diagnosis	Pyruvate carboxylase deficiency	Alpers syndrome	Leigh syndrome	Mitochondrial disorder

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P06.28D

Molecular assessment of variants in inherited lipodystrophy genes: prevalence and clinical impact in a large clinical care cohort

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Introduction: Inherited Lipodystrophies are disorders characterized by loss of adipose tissue accompanied by metabolic dysregulation. They are divided into Congenital Generalized Lipodystrophies (CGLs) and Familial Partial Lipodystrophies (FPLs). Lipodystrophies are considered rare genetic disorders with reported prevalences ranging from 1 in 10 million for CGLs, to 1 in 1 million for FPLs.

Methods: We interrogated the electronic health record (EHR) information for ~1.6 million individuals in the Geisinger Health System for lipodystrophy/lipoatrophy diagnosis codes. We performed genetic analyses of individuals with available genetic data from our Geisinger-Regeneron DiscovEHR collaboration to identify likely causative variants of lipodystrophy in these patients.

Results: Of 18 lipodystrophy-diagnosed patients with available genetic data, we identified potentially pathogenic variants in known genes in 8 individuals. Of these, 4 individuals carry the pathogenic variant in LMNA (p.R482Q) associated with Dunnigan FPL. There were additional 12 individuals harboring a molecular finding for the condition but no documented diagnosis of lipodystrophy.EHR review of these individuals showed that they have metabolic abnormalities consistent with lipodystrophy including diabetes, dyslipidemia, and pancreatitis. We surveyed the DiscovEHR cohort for pathogenic and likely pathogenic variants in lipodystrophy genes and evaluated the clinical and metabolic profiles of variant carriers. We observed a burden of metabolic dysregulation in these patients.

Conclusions: Our data show that partial lipodystrophy is an underdiagnosed condition and that its prevalence might be higher than previously reported.Carriers of lipodystrophy-associated variants show metabolic abnormalities in the spectrum of lipodystrophy disease, however they are often diagnosed with type 2 diabetes and unspecified metabolic syndrome.

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P06.29A

Clinical characterization of a novel (Asp116Gly) mutation in *PPARgamma* gene identified in a large Polish family

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Introduction: *PPARG* encodes peroxisome proliferator-activated receptor gamma, a nuclear hormone receptor which plays a crucial role in both glucose and lipid metabolism.

Materials and Methods: Genetic testing in a 44-year old female patient with familial partial lipodystrophy was performed by targeted NGS sequencing using a panel of 28 monogenic diabetes genes. Confirmation of the p.[(Asp116Gly)]; c.[347A>G] PPARG gene variant as well as verification of potential carriers in the family were subsequently performed using Sanger sequencing in 3130xl Genetic Analyzer. Clinical characteristics was subsequently assessed for the mutation carriers.

Results: Two programs were utilized in order to assess the impact of the detected missense variant on protein function: SIFT (Sorting Intolerant From Tolerant)[http://sift.bii.a-star.edu.sg/www/SIFT_intersect_coding_submit.html] and PolyPhen-2 (Polymorphism Phenotyping v2) [http://genetics.bwh.harvard.edu/pph2/]. These analyses supported the deleterious character of the mutation by revealing a PROVEAN score of -4,982. The amino acid position of 116Asp was also demonstrated to have a high degree of conservation between species. We confirmed this variant in 7 (out of 20) family members. They were diagnosed with diabetes, dyslipidemia, ischemic heart disease and suffered from myocardial infarctions before the age of forty. Additional family members have been recruited to further explore the impact of the mutation on the phenotype.

Conclusion: We assessed a family with autosomal dominant lipodystrophy related to a new PPARG mutation. The potential utility of NGS was confirmed for the

identification of families with rare forms of lipodystrophy, with the potential for consequently tailoring early treatment and prevention of IHD to prolong life expectancy.

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P06.31C

Development of an antisense-mediated exon skipping therapeutic strategy for Mucolipidosis II

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Lysosomal storage diseases (LSDs) are a class of inherited metabolic diseases caused by mutations in proteins critical for lysosomal function. Among them is ML II, which is caused by the deficiency of the GlcNAc-phosphotransferase, a key enzyme for the trafficking of lysosomal hydrolases to the lysosome. GlcNAc-phosphotransferase is encoded by two genes: *GNPTAB* and *GNPTG*. One of the most frequent mutations is a deletion on exon 19 of the *GNPTAB* gene that disrupts the reading frame impairing the production of an active enzyme and the targeting of lysosomal enzymes. Despite broad understanding of the molecular causes behind this and other LSDs, the same progress has not been observed in the development of therapies, with current treatments still mostly symptomatic. Therefore, alternative options should be investigated. One possibility is the modulation of splicing by antisense oligonucleotides (AOs) with the purpose of altering the mature mRNA and the final protein. This work intends to develop a RNA-based therapeutic agent through the use of AOs capable of inducing the skipping of exon 19 of the *GNPTAB* gene and circumvent the effects of this ML II mutation. Different 2'O-Methyl AOs were designed and tested. We have already succeeded in inducing the skipping of exon 19 in control and ML II patient fibroblasts. At biochemical level, 48 hours following transfection, enzyme activity suffered a small increase in patients fibroblasts for all enzymes tested, even if the results are still much lower than the observed for controls.

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P06.32D

Heterogeneity in m.3243A&gtG-related mitochondrial disease: The role of mtDNA heteroplasmy, copy number, age and nuclear factors

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Introduction: m.3243A>G, the most common pathogenic mitochondrial DNA (mtDNA) mutation, is associated with a range of clinical features, which progress at variable rates, making prognosis difficult to predict. We aimed to describe and understand the cause of this heterogeneity.

Methods: We examined the phenotypic profile of 238 m.3243A>G carriers from the UK MRC Mitochondrial Disease Patient Cohort using the Newcastle Mitochondrial Disease Adult Scale and evaluated which commonly assayed tissue (blood, urine, skeletal muscle) represents the m.3243A>G mutation load and mtDNA copy number most strongly associated with disease burden. We modelled the role of risk factors and additive nuclear genetic factors in the development of specific phenotypes within 46 pedigrees from the cohort.

Results: Age and m.3243A>G heteroplasmy level are associated with disease burden in all three tissues (R^2 range = 0.18-0.27, $P<0.001$); a greater proportion of the variation in disease burden is explained if mtDNA copy in skeletal muscle is included ($R^2=0.40$, $P<0.001$). Common phenotypic features include hearing impairment, psychiatric involvement and ataxia; age and heteroplasmy levels are poor predictors of phenotypic severity. We found high to moderate heritability estimates for psychiatric involvement, cognition, ataxia, migraine and hearing impairment (h^2 range = 0.40-0.76, $P<0.05$).

Conclusion: Our results indicate that m.3243A>G heteroplasmy, skeletal muscle mtDNA copy number and age explain some of the variation in m.3243A>G-related disease burden suggesting that nuclear genetic factors influence clinical outcomes, paving the way for future work identifying these.

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P06.34B

Analysis of mtDNA mutations in Serbian patients with Leber hereditary optic neuropathy

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Introduction: Leber hereditary optic neuropathy (LHON) is now regarded as the most frequent neurological disorder caused by mutations in a mitochondrial DNA (mtDNA), characterized by selective degeneration of retinal ganglion cells, optic atrophy, and central vision loss. Besides primary mutations number of secundary DNA changes affect phenotype. Aim of this study was to investigate the presence of mutations in mtDNA among Serbian patients affected with LHON.

Material and Methods: Individuals included in this study were recruited from the Child and Adolescent Neurology and Psychiatry Clinic and from Neurology Clinic CCS, Belgrade, Serbia. All examined individuals for 17 unrelated familes had characteristic clinical presentation suggesting the presence of LHON. Multiple segmental PCR amplicons of mtDNA have been sequenced by Sanger's method and the obtained results were compared with the referent mtDNA sequence.

Results: The most frequent primary mutations mt.3460 G>A in ND1 and mt. 11778 G>A in ND4 was found in 6 out of 15 and in 10 out of 15 families, respectively. LOHN primary mutation 11778 G>A was associated with ND1 mt.3394 T>C secondary mutation caused the evolutionary Conserved tyrosine to histidine (Y30H). The presence of both 11778 G>A and 3394 T>C mutations appear to contribute to higher penetrance of LHON. Many other mutations were detected in various parts of mitochiondrial genome. Some of haplotype variants were linked preferential to primary mutations.

Conclusion: Both primary and secondary mutations in mtDNA need to be better characterized in light of their association with clinical manifestations and progression of Leber hereditary optic neuropathy.

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P06.35C

Biallelic mutations in MRPS34 lead to instability of the small mitoribosomal subunit and Leigh syndrome

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The synthesis of all 13 mitochondrial DNA (mtDNA)-encoded protein subunits of the human oxidative phosphorylation (OXPHOS) system is carried out by mitochondrial ribosomes (mitoribosomes). Defects in the stability of mitoribosomal proteins or mitoribosome assembly impair mitochondrial protein translation, causing combined OXPHOS enzyme deficiency and clinical disease. Here we report four autosomal-recessive pathogenic mutations in the gene encoding the small mitoribosomal subunit protein, MRPS34, in six subjects from four unrelated families with Leigh syndrome and combined OXPHOS defects. Whole-exome sequencing was used to independently identify all variants. Two splice-site mutations were identified, including homozygous c.321+1G>T in a subject of Italian ancestry and homozygous c.322-10G>A in affected sibling pairs from two unrelated families of Puerto Rican descent. In addition, compound heterozygous MRPS34 mutations were identified in a proband of French ancestry; a missense (c.37G>A [p.Glu13Lys]) and a nonsense (c.94C>T [p.Gln32*]) variant. We demonstrated that these mutations reduce MRPS34 protein levels and the synthesis of OXPHOS subunits encoded by mtDNA. Examination of the mitoribosome profile and quantitative proteomics showed that the mitochondrial translation defect was caused by destabilization of the small mitoribosomal subunit and impaired monosome assembly. Lentiviral-mediated expression of wild-type MRPS34 rescued the defect in mitochondrial translation observed in skin fibroblasts from affected subjects, confirming the pathogenicity of MRPS34 mutations. Our data establish that MRPS34 is required for normal function of the mitoribosome in humans and furthermore demonstrate the power of quantitative proteomic analysis to identify signatures of defects in specific cellular pathways in fibroblasts from subjects with inherited disease.

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P06.36D

Mutations in *NDUFAF8* cause Leigh syndrome with an isolated complex I deficiency

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Introduction: Mitochondrial diseases are clinically and genetically heterogeneous metabolic conditions. Complex I deficiency is the most common biochemical diagnosis, particularly prevalent in paediatric patients. Mitochondrial disease is caused by mutations affecting the mitochondrion's own genome (mtDNA) or one of the ~1150 nuclear-encoded mitoproteome components. The limited genotype: phenotype correlation in mitochondrial disease means next-generation sequencing methodologies are critical for the rapid genetic diagnosis of patients.

Materials and Methods: Whole exome and targeted next-generation sequencing was performed for three

subjects with suspected mitochondrial disease. Functional evaluation of identified variants was undertaken using subject fibroblasts and/or muscle biopsy, including cDNA studies, complexome profiling, complementation studies and assessment of steady-state levels.

Results: We describe three unrelated individuals who harbour biallelic variants in *NDUFAF8*, a recently identified ancillary factor required for assembly of the complex I holoenzyme. We provide functional evidence to support the pathogenicity of these *NDUFAF8* variants and unequivocally establish this gene as a cause of complex I deficiency in association with an exclusively Leigh-like clinical presentation.

Conclusions: Functional experimentation including complementation studies and complexome profiling of subject cell lines establishes *NDUFAF8* as the twelfth complex I assembly factor associated with human disease and validates the importance of orphan gene characterisation.

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P06.37A

Unexpected genetic diagnosis of mitochondrial disease in three consanguineous Turkish families

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Introduction: Mitochondrial diseases are a clinically heterogeneous group of disorders caused by dysfunction of the mitochondrial respiratory chain. Some mitochondrial disorders affect a single organ, while many involve multiple systems such as skeletal muscle, brain, heart and liver, leading to diagnostic difficulties. Here we present three patients who were originally suspected to have a primary disease of skeletal muscle, leukodystrophy and brain malformation.

Materials and methods: Patients were recruited from three paediatric neurology clinics in Turkey: Izmir, Malatya and Diyarbakir. Whole exome sequencing (WES) was performed using Illumina exome capture (38 Mb target). Data analysis was carried out on the RD-Connect Genome-Phenome Analysis Platform. Standard filtering criteria with MAF<1% and high/moderate VEP were used, as well as a list consisting of >5,000 medically interpretable genes.

Results: We identified a homozygous frameshift variant (p.Glu41GlyfsTer10) in *NDUFA12* and a homozygous missense variant (p.Gln85His) in *NDUFS3*, both associated with Leigh syndrome due to mitochondrial complex I deficiency (OMIM# 256000), and a homozygous nonsense variant (p.His158ProfsTer8) in *TACO1* associated with mitochondrial complex IV deficiency (OMIM# 220110). All the variants were highly pathogenic and were absent in the control population, suggesting they were disease-causing. Critical clinical review and metabolic analysis confirmed the mitochondrial deficiency.

Conclusions: Next generation sequencing has the advantage of allowing an unbiased genetic diagnosis. We described three cases that had been initially diagnosed as myopathy, brain malformation and leukodystrophy, and WES resulted in the diagnoses of mitochondrial disorders. Importantly, this will allow for appropriate clinical management of these patients.

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P06.38B

Innovative Next Generation Sequencing strategy for screening mitochondrial DNA mutations as a robust alternative of conventional methods

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Human mitochondria produce ATP and metabolites to support development and maintain cellular homeostasis.

Mitochondria harbor multiple copies of a maternally inherited non-nuclear genome (mtDNA) and defects in its replication or nucleotide metabolism cause point mutations, deletions, or depletion of mtDNA. These genetic alterations can lead to multi-system syndromes, including neuromuscular and neurodegenerative diseases. To date, approaches based on Sanger technology and Southern blot have formed the basis of mtDNA screening but these technologies are inherently hampered by limitations in speed, throughput, resolution, and associated costs.

In this study, we describe a robust strategy for screening mtDNA alterations through a PCR free NGS approach in order to assess with high accuracy point mutations and single or multiple large deletions in both homoplasmic or heteroplasmic state. Mitochondrial DNA is extracted from biological samples with the Mitochondrial DNA Isolation Kit (Abcam), processed according to Nextera XT DNA library Prep Kit (Illumina), and sequenced on MiSeq benchtop sequencer (Illumina). The output obtained for each sample analyzed is represented by the full sequence of mtDNA at an high depth of coverage. We developed an innovative bioinformatic analysis of deep NGS data to detect mitochondrial haplogroups, heteroplasmic mutations and large mtDNA deletions with higher accuracy and sensitiveness respect to standard techniques. In addition, this approach allows us to precisely map and quantify large heteroplasmic mtDNA deletions from the analysis of NGS coverage data, avoiding the bias introduced by polymerase amplification for shorter mtDNA molecules that characterize the NGS approaches published in the literature so far.

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P06.39C
Diagnosis of mitochondrial disorders by whole exome sequencing

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Introduction: The Malta BioBank (BBMRI.mt) participated in the BBMRI-Large Prospective Cohort (BBMRI-LPC) whole exome sequencing (WES) call with a collaborative research project with Hacettepe University, Turkey to sequence a total of 50 exomes from patients with genetically undiagnosed mitochondrial disorders.

Materials and Methods: The Maltese cohort included 13 probands (7 children and 6 adults) and 2 unaffected relatives. Whole exome sequencing and bioinformatics analysis were carried out at the Centro Nacional de Análisis Genómico (CNAG-CRG) in Barcelona. Phenotypic data of each participant was recorded on PhenoTips. Exome data was analysed on the RD-Connect Genome-Phenome Analysis Platform.

Results: A comparative analysis of rare autosomal recessive mutations shows that some patients share the same variants. Rare missense mutations in the mitochondrially encoded cytochrome B gene (MT-CYB) at positions 14766 and 15326 were present in 6 and 11 of the probands respectively. The mtDNA mutation at position 15326 (rs2853508) was not present in the reference Maltese Exome database, whereas that at position 14766 (rs57236041) had a frequency of 59%.

Conclusion: The exome sequence data generated through this collaborative research will aid in establishing a genetic diagnosis for these rare disease patients.

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P06.40D
High-throughput sequencing of the whole mitochondrial genome in 974 patients with mitochondrial disease: New insights and challenges for the interpretation of mitochondrial DNA variants

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Mitochondrial diseases owe their clinical heterogeneity to the dual origin of mitochondrial proteins: nuclear and

mitochondrial genomes (mtDNA). We used whole mtDNA analysis by Next Generation Sequencing (NGS) in a cohort of 974 patients with suspected mitochondrial disease. Our study demonstrated the power of our strategy, especially using uroepithelial cells as source of mtDNA, with the identification of a pathogenic variant in 130 patients (13.3%). However, massive parallel sequencing used raised several issues such as: **1. Low pathogenic mtDNA variant loads**. The identification of low mutation rates contributes to improved diagnosis, especially among relatives. However, evaluating the clinical relevance of these low mutation rates in probands is complex, as for the m.3243A> G, detected at low levels for 16 patients.**2. Pathogenic variants unrelated to patient phenotype**. Nevertheless, some of them could be regarded as "actionable mutations" calling for specific recommendations in patient management.**3. Variants of unknown significanc**e. A novel variant was identified in 3.7% of the patients highlighting the difficulty in prioritizing them, because of the weakness of *in silico* tools and databases, and the lack of guidelines.**4. Integrative analysis**. Currently, diagnostic laboratory do not use information such as mitochondrial haplogroups or rare co-occurrences of mtDNA variants, for explaining phenotypic differences within patients carrying the same mutation. Our study shows that mtDNA screening by NGS significantly improves the diagnosis of mitochondrial disorders. However, the technique increases the complexity of appreciating the variants identified, thereby posing new challenges in the molecular diagnosis of mitochondrial diseases.

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P06.42B

Mutations in the mitochondrial intermediate peptidase (*MIPEP*) cause multiple OXPHOS deficiency, psychomotor retardation, cerebellar syndrome and axonal neuropathy

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Mitochondrial diseases represent a large group of rare and heterogeneous genetic disorders that are associated with different disease mechanisms.

Here, we report an abnormal processing of precursor proteins in human mitochondria by the mitochondrial intermediate peptidase (MIPEP) leading to mitochondrial disease. Most nucleus-encoded mitochondrial proteins are synthesized in the cytosol as precursor proteins carrying an N-terminal targeting signal that is cleaved upon import into mitochondria by the matrix processing peptidase (MPP) and in some cases by other processing peptidases, like MIPEP to ensure protein stability and functionality. Using exome sequencing, we identified mutations in the *MIPEP* gene in a patient presenting psychomotor retardation, cerebellar syndrome and axonal neuropathy. MRI shows hyperintensities in the bilateral atrophic putamen.

Western immunoblotting of patient fibroblast extracts revealed strong decease of the mutant MIPEP protein and an accumulation of the unprocessed precursor of MRPL12 – a bona fide MIPEP substrate. Analysis of the assembly and function of OXPHOS using blue-native (BN) PAGE and respirometry, respectively, showed defects in the biogenesis and activity of complexes I, IV and V in subject's fibroblasts. We carried out functional complementation experiments in patient fibroblasts stably expressing wild type MIPEP protein, which showed restoration of MRPL12 processing and OXPHOS assembly and function thereby confirming that *MIPEP* is the disease-causing gene. Our studies further expand the genotypic and phenotypic heterogeneity of MIPEP-linked mitochondrial disease and provide important insights into its pathophysiology.

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P06.43C

Mutations in *MRPS14* cause intellectual disability, neonatal lactic acidosis, cachexia and hypertrophic cardiomyopathy with distinct dysmorphic features

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Introduction: Multiple respiratory dysfunction is associated with defects in mitochondrial replication and

translation. Possibly, due to their detrimental effect, defects of the mitochondrial ribosome are rare. Here we report that a mutated essential mitochondrial small ribosome subunit causes mental retardation, cachexia, muscle hypotonia, hypertrophic cardiomyopathy with elevated lactate in a Turkish girl born to consanguineous parents.

Materials and Methods: DNA from blood was used for Next Generation Sequencing via MitoExome panel. Mitochondrial respiratory chain function was assessed in skeletal muscle via enzymatic activity and in primary fibroblasts via oxymetric measurements and native mitochondrial complex assembly. Ribosomal RNA stability was analysed by Northern-blotting and qPCR in fibroblasts. Mitochondrial translation capacity was determined in fibroblasts by ^{35}S-methionine incorporation into newly synthesized proteins and separated on SDS-PAGE. For complementatio, wild-type *MRPS14* cDNA was cloned into a lentiviral vector and stable lines generated via antibiotic selection.

Results: Targeted next generation sequencing revealed a homozygous variant in mitochondrial small ribosomal protein 14 (*MRPS14)*. Biochemical characterisation revealed an enzymatic complex IV deficiency in skeletal muscle with total mitochondrial translation greatly decreased in fibroblasts alongside all mitochondrially encoded respiratory subunits. Lentiviral complementation confirmed the pathogenicity of the novel variant.

Conclusions: This is the first mutation in any mitochondrial ribosomal protein so far reported not affecting ribosomal stability, but drastically decreasing mitochondrial translation capacity.

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P06.44D

Recessive mutations in *UQCRFS1*, encoding the Rieske iron-sulfur protein, are associated with mitochondrial complex III deficiency, lactic acidosis and cardiomyopathy

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Introduction: Complex III (CIII) presents the center of the mitochondrial respiratory chain. Its deficiency is one of the least common oxidative phosphorylation defects associated with mitochondrial disease, with pathogenic variants in 10 genes encoding complex III subunits or assembly factors identified and associated with a broad phenotypical spectrum.

Material and methods: Two unrelated individuals with early-onset hypertrophic cardiomyopathy and lactic acidosis underwent genetic analysis (whole exome sequencing, RT-PCR, Sanger sequencing). Functional studies were performed in proband derived fibroblasts.

Results: We identified biallelic mutations in UQCRFS1, encoding the ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1, a catalytic subunit of the CIII. One proband carried homozygous intronic splice-site variant. Splicing analysis revealed deletion of 30 nucleotides. The other proband had two compound heterozygous missense variants. Proband derived fibroblasts showed deficient oxygen consumption rate. Western blot analysis of isolated mitochondria showed a reduction of UQCRFS1 in affected probands, as well as a decrease of CIII assembly factor UQCC2. We also observed reduction of complex I subunit NDUFS4. Furthermore, Blue-Native gel electrophoresis of digitonin-solubilized mitochondria showed decrease of both complex III and complex I.

Conclusions: Until now, variants in UQCRFS1 and impaired complex III activity have only been reported in relation with gastric and breast cancer. Here we present evidence that biallelic variants in UQCRFS1 can cause mitochondrial disease with early onset cardiomyopathy and lactic acidosis. Combined complex III/I deficiency seems to be the common pattern of complex III subunit/assembly factor defects.

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P06.45A

Homozygosity mapping in maple syrup urine disease patients from Iran: Identification of novel, recurrent mutations and in silico analysis of novel mutations

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Introduction: Maple syrup urine disease (MSUD) is a rare inborn error of metabolism of branched-chain amino acid metabolism. The disease prevalence is higher in populations with the higher rate of consanguineous marriage like Iran (38.6%). Different mutations have been previously reported in BCKDHA, BCKDHB, DBT, and DLD is known to be responsible for MSUD phenotype.

Materials and Methods: In this study, two sets of multiplex polymorphic STR (short tandem repeat) markers linked to the above-mentioned genes were used in homozygosity mapping in order to find probable pathogenic changes in 40 studied families. The families who showed homozygous haplotypes for BCKDHA, BCKDHB and DBT genes were subsequently sequenced.

Results: Our findings revealed that exon 2, 4 and 6 of BCKDHA gene contained most of the mutations which were novel. The changes include one reported point mutation (c.890G>A (p. R297H)), 7 nucleotide insertion (c.355-356 Ins 7nt (p. D355D fs)) and a splice site mutation (c.288G>A). In BCKDHB gene we identified one reported (c.853 C>T (p. R285X)) and two novel point mutations [(c.599 C>T (p. P200L), c.484 A>G (p. N162D)]. In DBT gene we found novel homozygote deletion of exon 5,6 and 7 in one patient as well as a point mutation and deletion (c.363delCT/ c.1238T>C).

Conclusion: Computational approaches were used to analyze these novel mutations in terms of their impact on protein structure. Computational structural modeling indicated that these mutations might affect structural stability and multimeric assembly of branched-chain keto acid dehydrogenase complex (BCKDC).

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P06.46B

Exome sequencing can reliably identify mtDNA variants increasing the diagnostic yield in a diagnostic context

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Mitochondrial diseases can result from mutations in either the mitochondrial DNA (mtDNA) or the nuclear DNA (nDNA). The most commonly used techniques to detect single nucleotide variants in the mtDNA are Sanger sequencing of certain mutations or next generation sequencing of the whole mtDNA. With the introduction of exome and genome sequencing, it is now possible to detect variants on both the nDNA and the mtDNA with one comprehensive approach. We performed an analysis of the mtDNA on exome sequencing data from leucocyte derived DNA of 1,692 individuals for which exome sequencing was performed in a diagnostic setting with various indications. In total, 26 (likely) clinical relevant variants in the mtDNA were detected. This resulted in 40 conclusive diagnoses equaling an overall diagnostic yield of ~2.4%. Looking only at the solved cases, 5.7% had disease due to mutations in the mtDNA. Of the 26 different variants, three were listed as "reported" in MitoMap and we confirmed pathogenicity by functional tests and/or segregation analysis. An additional eight novel, to date unpublished variants were identified. Of note, we did not only identify causative mutations in patients with suspected mitochondrial disease. However, read-depth of the mtDNA was not enough to reliably detect deletions in the mtDNA and exome sequencing should be complemented by e.g. long-range PCR in certain cases. This study shows that mutations in the mtDNA can be found in a significant proportion of patients with suspected monogenic disorders. Exome sequencing can reliably detect those variants resulting in a high diagnostic yield.

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P06.47C

Common facial phenotype of patients with Mucolipidosis type IV: a clinical observation reaffirmed by facial dysmorphology novel analysis technology

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Background: Mucolipidosis type IV (ML-IV) is a rare autosomal recessive lysosomal storage disease, caused by mutations in the *MCOLN1* gene. It manifests with non-specific symptoms of developmental delay, esotropia and even corneal clouding. While the clinical phenotype, molecular basis and underlying pathomechanism have been

described, the diagnosis of ML-IV remains elusive and patients are often misdiagnosed. Our clinical observation was that ML IV patients share common and identifiable facial features, which have yet to be included in the clinical phenotype as described in the literature to date. Objective and methods: In order to validate these findings using an objective and digital tool, two-dimensional facial images of ten patients with ML-IV, obtained at various ages, were analyzed using facial dysmorphology novel analysis (FDNA). This technology utilizes various measurements extracted from automatically-detected facial points from facial photographs, to recognize distinct dysmorphic features and analyze their similarities to known facial patterns, termed gestalts.

Results: When analyzed in comparison to a control cohort of unaffected cases (n = 100) and a cohort of cases diagnosed with syndromes other than ML-IV (n = 100), the ML-IV cohort showed a mean area-under-the-curve (AUC) of 0.77 (SD, 0.19) and 0.87 (SD, 0.05), respectively.

Conclusions: We describe for the first time recognizable facial features typical in patients with ML-IV. Reaffirmed by the objective FDNA technology, the described common facial gestalt adds to the tools currently available for clinicians and may thus assist in reaching an earlier diagnosis of this rare and underdiagnosed disorder.

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P06.48D
Molecular Characterisationof MPS IVA patients in Andean region of Colombia

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Introduction: Mucopolysaccharidoses (MPS) are a group of inherited metabolic lysosomal storage disorders. A subgroup of this is Morquio disease, an autosomal recessive condition which overall incidence is 0.68 per 100,000 live births. In Colombia, studies suggest that MPS IVA is likely the highest prevalence worldwide.

Materials and Methods: Sixteen families and nineteen patients from a different region of the country were tested for mutation identification, the sequence was compared to the GALNS reference sequence NM_000512.4, and gene variants were reported. Bioinformatics analysis was done by SWISS-MODEL, the mutant proteins were generated by homology from the wild-type GALNS 4FDI template obtained from PDB database and visualization was performed using Swiss-PdbViewer. The predictive analysis was run in PolyPhen-2 software (Polymorphism Phenotyping v2) and SIFTS human protein v1.03 software.

Results: 79% of the cohort was homozygous and 21% were compound heterozygous. The mutation c.901G>T was the most frequent mutation with 74% of the alleles 10,5% followed by mutation c.1156C>T. In addition, 1 novel mutation was described in c.214T>A predictive analysis identify it as pathogenic variant.

Conclusion: This study reveals the mutation spectrum of MPS IVA in the Colombian population. The mutation spectrum data for MPS IVA disorder in the Colombian population are not yet completely characterized. The high prevalence of the c.901G>T mutation suggest it is a founder effect cause diseases in this particular region, and In addition as a migration process in the Andean region. This spectrum data will be useful in the provision of better genetic counseling, and prenatal diagnosis.

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P06.49A
Biallelic missense and deep intronic *NDUFAF6* variants, unraveled by exome sequencing and mRNA analysis, in patients with Leigh syndrome

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Introduction: NADH dehydrogenase complex I assembly factor 6 (*NDUFAF6*) gene encodes a mitochondrial protein which is essential for early assembly stages of mitochondrial respiratory complex I. Biallelic mutations in *NDUFAF6* have been identified as responsible for cases of autosomal recessive Leigh syndrome associated with mitochondrial complex I deficiency. Patients and Methods: we studied two siblings and two unrelated subjects with Leigh syndrome. Whole exome sequencing (WES), quantitative PCR and sequencing on NDUFAF6 transcript were performed on patients' biological specimens

Results: By WES, we found a variant in *NDUFAF6* (c.532G>C:p.A178P) in two siblings and a singleton unrelated subject, all affected by Leigh syndrome. The same missense mutation was recently described in a patient presenting Leigh syndrome and complex I deficiency, associated with an almost monoallelic expression of the mutated allele at transcriptional level; nevertheless, the second pathogenic mutation remained unidentified. Here we provide evidence that the second allelic mutation consists of a deep intronic variant present in all affected individuals, including the previously published case. Through mRNA analysis we demonstrated that the identified intronic mutation is responsible for the formation of an alternative splice site, leading to production of an aberrant transcript.

Conclusions: A detailed analysis of whole exome sequencing data together with functional validation based on mRNA analysis may reveal pathogenic variants even in non-exonic regions. Acknowledgments: Telethon Grant GGP15041; Mariani Foundation; MRC-QQR (2015-2020) grant; the ERC advanced grant FP7-322424, NRJ-Institut de France grant; Italian Ministry of Health. The Telethon Biobank (grant GTB12001J) supplied biological specimens.

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P06.50B

Integration of research within clinical care identifies 14 novel genetic causes of neonatal diabetes

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Neonatal diabetes (NDM) is diagnosed before 6 months of age and has >25 known genetic causes. Since 2000 the Exeter laboratory has offered free genetic testing to anyone diagnosed with NDM. Testing of all known genetic causes of NDM using Sanger sequencing, methylation-specific-MLPA and targeted next-generation-sequencing is performed by the Exeter Genetics laboratory using the same pipeline and quality parameters of diagnostic tests. Patients without a genetic diagnosis are tested by whole-exome/genome sequencing as part of the novel genetic aetiologies research study.

We assessed the success rate of this approach in terms of 1) the proportion of patients with a genetic diagnosis identified; 2) how many novel genetic causes were found.

1673 NDM patients referred from 93 countries had comprehensive genetic testing. 1484 individuals (88.7%) had a genetic diagnosis identified, resulting in improved treatment for 620 patients.

Further testing by whole-genome (N=69) or whole-exome (N=24) sequencing defined the genetic diagnosis in 34 individuals and identified 14 novel causes of NDM. Eleven patients had pathogenic variants in disease genes for which neonatal hyperglycaemia had not been previously reported as part of the phenotype (*COQ2, COQ9, LPL, OXCT1, NARS2, TARS2*). Pathogenic variants in four genes thought to cause diseases unrelated to NDM were found in 69 patients in our cohort (*GATA6, STAT3, LRBA, WFS1*). Four novel disease genes were identified in 14 patients.

Our integrated diagnostic and research approach defined the genetic cause of NDM in 88.7% of patients (with improved treatment for 19%) and identified 14 novel genetic causes of NDM.

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P06.51C

Targeted next generation sequencing utility in diagnosis of complex metabolic and neurological disorders

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Introduction: Metabolic and neurological conditions are a huge group of disorders and syndromes, characterized by clinical variability and extreme genetic heterogeneity. Targeted next generation sequencing (tNGS) provides possibility for precise genetic diagnosis of metabolic and neurological disorders.

Materials and methods: Six patients with metabolic and/or neurological symptoms were referred in 2016/2017 to the Laboratory of Genome Diagnostics for performing tNGS. The analysis was implemented on MiSeq Illumina with TruSight One kit. Resutls: In one of the patients with neurodegenerative disease and congenital defect in glycosylation we did not find clinically relevant variants leading to the observed phenotype. In two of the patients with

clinical diagnosis glycogenosis type IXa and methylmalonic acidemia genetic testing confirmed the diagnosis and we found mutations in *PHKA2* and *MMAA* respectively. The mutation in *PHKA2* was new frameshift variant. In two patients with epilepsy the genetic testing clarified the diagnosis. In one of these patients homozygous pathogenic mutation leading to severe reduction of *PRODH* activity was found. The test changed the diagnosis from epilepsy to hyperpolinemia, type I. In the second patient with epilepsy and progressive muscular hypotonia a new pathogenic frameshift variant in *SLC16A2* gene mutation was found leading to Allan-Herndon-Dudley syndrome.

Conclusions: Our results prove that tNGS is cost effective and efficient method, which allows a more precise diagnosis to be made for many complex disorders and could be considered earlier in the diagnostic practice. Acknowledgements**:** The research was supported by grant DUNK01-2/2009 by NSF, Ministry of Education and Science.

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Persistent hypoglycemia in children: contribution of NGS in diagnosis of inborn errors of metabolism

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Introduction: Hypoglycemia is an important cause of pediatric morbidity and is often due to inborn errors of metabolism (IEM) that present with clinical, biochemical and genetic heterogeneity. We have developed a rapid and accurate strategy for the molecular diagnosis of hypoglycemia- associated IEMs.

Materials and Methods: 64 patients were tested through a custom gene panel of 65 genes, which included five disease categories: hyperinsulinemic hypoglycemia (HI), fatty acid-oxidation (FAOD) and ketogenesis defects, ketolysis defects, glycogen storage diseases (GSDs) and other disorders of carbohydrate metabolism, and mitochondrial disorders. Molecular data were compared with clinical and biochemical data. Patients, investigated through an extensive workup, were divided in 3 diagnostic classes: a) single candidate gene (SCG-9/64), b) multiple candidate genes (MCG-43/64) and c) no candidate gene (NCG-12/64).

Results: A proven diagnosis was obtained in 78% of patients in SCG, in 49% in MCG and in 33% in NCG. The diagnostic yield was 48% for HI, 66% per FAOD and ketogenesis defects, 59% for GSDs and other carbohydrate disorders, and 67% for mitochondrial disorders.

Conclusions: This approach provided a diagnosis in about 50% of patients in whom clinical and laboratory evaluation did not allowed to identify a single candidate gene, overtaking genetic heterogeneity and clinical variability. Remarkably, a diagnosis was established in 33% of patients belonging to the no candidate gene class. Our study shows that NGS technique is cost-effective compared to Sanger sequencing of multiple genes, and represents a powerful tool for the diagnosis of inborn errors of metabolism presenting with persistent hypoglycemia

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Polymorphisms in *PPARG* gene: association with obesity-related metabolic traits in a Serbian adolescent population

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Introduction: The *peroxisome proliferator-activated receptor γ (PPAR-γ)* is a candidate gene for obesity and type 2 diabetes mellitus. Protein product PPAR-γ is a lipid-activated transcription factor that has a main role in the expression of genes involved in adipocyte differentiation and function. Although the association of a common single nucleotide polymorphism (SNP) of the *PPAR-γ* gene, Pro12Ala (34C>G, rs1801282), with obesity has been reported in various populations, these data are not conclusive. Similarly, conflicting results, were obtained regarding the association between metabolic phenotypes and another frequent variant, a silent mutation of the *PPAR-γ* gene, His477= (1431C>T, rs3856806).

This study aimed to estimate whether the *PPAR-γ* rs1801282 and *PPAR-γ* rs3856806 SNPs are linked with obesity-related metabolic traits in a Serbian adolescent population.

Materials and Methods: Anthropometric and biochemical parameters were measured in 84 adolescent patients at the age of 15, whose BMI was over 85th percentile. Body mass index (BMI), fasting glucose, triglyceride, systolic and diastolic blood pressure and total cholesterol were measured. Subjects were genotyped for Pro12Ala and 1431C>T

SNPs by PCR-restriction fragment length polymorphism analysis.

Results: The Pro12Ala variant was associated with higher diastolic blood pressure in male subjects carrying G allele ($p = 0{,}041$). The 1431C>T variant was associated with higher systolic blood pressure in male subjects carrying T allele ($p = 0{,}002$).

Conclusions: Results of our preliminary study indicate that a rare variant Pro12Ala (allele G), and 1431C>T rare variant (allele T) appear as risk factors for higher diastolic and systolic blood pressure, respectively, in overweight and obese male adolescents.

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P06.54B

Functional genomics' studies are mandatory for clarifying pathogenicity of novel genetic variants detected by NGS in OXPHOS disorders

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Introduction: Genetic causes of OXPHOS disorders include mutations in mitochondrial and/or nuclear genomes. With the significant increase of genetic diagnoses following NGS techniques, demonstrating the pathogenicity of novel variants became a challenging issue.

We present 3 patients with novel genetic variants in whom functional studies were mandatory for illuminating pathogenicity.

Case reports: Patient 1. Leigh syndrome (40y, male); complex IV deficiency (29.7% activity) and novel homozygous deletion (c.-11_13del, *SURF1* gene) [*Ribeiro et al. Mitochondrion31(2016)84–88*], detected by Sanger sequencing and not by NGS, demonstration of complex IV assembly (fibroblasts).

Patient 2. Epileptic encephalopathy (8y, male); moderate OXPHOS decrease and novel detected homozygous mutation (*FASTKD2*). Functional analyses revealed decreased protein expression and respiratory rate/ATP production, with increased glycolytic capacity (fibroblasts).

Patient 3. Case of CPEO (62y, female); multiple OXPHOS deficiencies (muscle) and two mtDNA alterations (m.7486G>A, *MT-TS1;* mt-tRNA$^{Ser(UCN)}$, 4,977bp deletion) [*Bacalhau et al. Neuromuscular Disorders, in press*]. Diverse functional studies unveiled the energy failure cellular causes.

Conclusion: We present three cases of OXPHOS diseases due to different genetic causes: 1 novel nuclear mutation in a gene affecting a known protein (surfeit 1, assembly factor of complex IV); 1 novel nuclear mutation in a gene affecting a recently known protein; 2 mtDNA alterations (1 known, 1 unclassified). Limitations of NGS in mutation detection and evaluation of OXPHOS activity as the only functional parameter are addressed.

In all cases, the functional genomics' approach was essential for clarifying pathogenicity, with implications for genetic counselling.

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P06.55C

Sequential approach to identify disease causing variants in patients with mitochondrial dysfunction of a Hungarian cohort

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Genetic diagnosis of disorders with mitochondrial dysfunction is often challenging due to the phenotypic variability and considerable genetic heterogeneity. Here we would share our experiences on analysis of 192 individual WES data using phenotype-driven prioritization or candidate genes-based filtering. Patients with positive family history were enrolled if targeted genetic tests failed to yield conclusive results. Multisystemic and neurodegenerative diseases were preferred with high-quality health records. Phenotype profiles were reconstructed from clinical documentation using HPO (#14.5+/-10.7terms/patient). The

selected cases were classified into disease-groups based on the main presenting symptoms: 27%mitochondrial/multisystemic disease, 12%early-onset Parkinson, 7%dementia and 11%NBIA/dystonia syndromes. Besides these groups, 24% unclassified and 19% were used as unaffected relative or healthy control; 128 as singletons, 64 individuals as members of 26 examined families were analyzed, respectively. WES was performed on Illumina HiSeq2500 platform. For interpretation, the Exomiser algorithm was applied at first, using variant and HPO descriptors, to rank variants based on predicted pathogenicity and semantic similarity to known phenotypes. Secondly, multi-gene-panel analysis was focused to disease-related genes that are presumptive to the pathogenesis (e.g. neurodegeneration). The ranked variants were further filtered using in-house data warehouse. After screening known causal variants of candidate genes a large number of annotated features were considered to fine-tune the hypothesis about causality of a given variant to reach cc. 30% diagnostic rate. To assess candidates, item-level similarity of phenotypic terms and reverse-phenotyping of hallmark symptoms were often needed to complement clinical data with the typical, but routinely not tested manifestations of the possible inherited disorder.

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P06.56D

Genotype-phenotype correlations and BH_4 predicted responsiveness in patients with phenylketonuria from Rio de Janeiro, Southeast Brazil

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Introduction: The clinical phenotypes of phenylketonuria (PKU) are highly variable. This has been attributed to genetic heterogeneity and frequent compound heterozygosis. The correlations between phenotypic characteristics, BH_4 predicted responsiveness, and the causative mutations found in PKU patients from Rio de Janeiro, Brazil, were evaluated.

Materials and Methods: A total of 102 completely genotyped patients were included. They were assigned to one of the following phenotypes according to pre-treatment phenylalanine (Phe) levels: classic, moderate and mild PKU, and mild hyperphenylalaninemia (MHP). For each genotypic combination, the predicted phenylalanine hydroxylase (PAH) residual activity (PRA) and the sum of arbitrary assigned values (AV) were determined. Genotype-based predictions of responsiveness to BH_4 were also analyzed.

Results: A strong relationship between genotypic severity, according to the level of PRA, and the inverse of pre-treatment Phe levels was observed ($t = 4.79$, $P<0.0001$). The observed phenotype matched the AV predicted phenotype in 48% of the cases. A BH_4-responsiveness rate of 37.0% was estimated.

Conclusions: The high degree of discordance found when the AV sum prediction system was employed fell to 33% once the phenotypes reported at the BIOPKU database were also considered. We estimated that 81% of the patients were potential candidates for a BH_4 loading test. Analyzing the functionally hemizygous patients, we found that the moderate common mutations, p.R261Q, p.V388M, and p.I65T contributed decisively to the high genotype-phenotype discordance. Despite these discrepancies, genotype continues to be the main determinant of metabolic outcome in most patients with PKU, anticipating their dietary needs and BH_4 responsiveness.

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P06.57A

Mutation analysis of the *PAH* gene in phenylketonuria patients from Rio de Janeiro, Southeast Brazil

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Phenylketonuria (PKU) is an autosomal recessive disease resulting from mutations in the *PAH* gene. Most of the patients are compound heterozygotes, and the combination of mutations is a major factor in determining the phenotypic differences found among them. The mutational spectrum of PKU in the state of Rio de Janeiro, Southeast Brazil, was analyzed by sequencing the *PAH* gene from genomic DNA of 102 patients. Deletions and duplications were also screened using MLPA analysis. Both mutated alleles were identified in all patients. Nine (8.8%) homozygous and 93 (91.2%) compound heterozygous patients were found. The spectrum included 37 causative mutations, including a new mutation - p.G312C. Missense, nonsense, and splicing variants corresponded to 63.7%, 2.9% and 22.6% of the mutant alleles, respectively. Large (1.5%), and small deletions, inframe (5.4%) and with frameshift (3.9%), comprised the remainder. The most frequent mutations were: p.V388M (12.7%), p.R261Q (11.8%), IVS10-11G>A (10.3%), IVS2+5G>C (6.4%), p.S349P (6.4%), p.R252W (5.4%), p.I65T (4.4%), and p.T323del (4.4%). The Iberian Peninsula, especially Portugal, is the major source of *PAH* mutations in Rio de Janeiro. Mutations that have other geographical origins, such as IVS2+5G>C, p.G352Vfs*48, and IVS12+1G>A were also detected. Genetic drift and founder effect may be responsible for the high frequencies of the mutations p.S349P and p.T323del in this population. Grant from "Coordination for the Improvement of Higher Level Personnel (Capes) of the Ministry of Education, Brazil'

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P06.58B

Molecualr genetics of a cohort of more than 770 cases of PKU in a consanguineous population

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Phenylketonuria (PKU) is an inborn error of amino acid metabolism caused by mutations in phenylalanine hydroxylase (PAH). Herein, we reported that among the 772 patients diagnosed with PKU, there are 635 classic (82%) and 137 previously reported non-classic subtypes (18%) from all ethnicities of 31 provinces in Iran. The disease causing mutations were found in 611 out of 635 classic (with a diagnostic detection rate of 96%) and in 97 out of 137 non-classic patients (with a diagnostic detection rate of 71%). To the best of our knowledge, this report is the most comprehensive study of the molecular genetics of PKU in Iran, identifying 100 distinct mutations in the PAH gene, including 15 previously unreported mutations. Interestingly, we found unique cases of PKU with uniparental disomy, germline mosaicism and co-inheritance with another Mendelian single-gene disorder that provides new insights for improving the genetic counseling, prenatal diagnosis (PND) and/or pre-implantation genetic diagnosis (PGD) for inborn error of metabolism group of disorders.

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P06.59C

PMM2-CDG patients gestalt: is recognizable enough?

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Background: Phosphomanomutase deficiency (PMM2-CDG, MIM#212065) is the most frequent congenital disorder of glycosylation. Initially PMM2-CDG was defined as a combination of systemic involvement, developmental delay due to cerebellar atrophy, peculiar fat pads and inverted nipples. It seems to be mild and not specific dysmorphic facial features that may change with age. We aim to describe the dysmorphic facial traits and draw a recognizable facial pattern.

Methods: We evaluated the frequency of occurrence of clinical symptoms and analysed the performance of facial gestalt computer-assisted image analysis in PMM2-CDG patients. We used the Research application of the Face2Gene tool (FDNA Inc. Boston, MA, USA). It generated a classification model on three groups of frontal photos: the group-PMM2, unaffected and Angelman Syndrome, because it was the most frequent syndrome-match offered by the automated tool.

Results: We included 34 patients, 20 boys and 14 girls, aged 6-18, of Caucasian ethnicity. The confusion matrix describing a multiclass comparison of actual class as compared to a predicted class showed a true positive rate for each group with values significantly better than a random assignment of photos. The area under the receiver operating characteristics curve (AUC of ROC) demonstrated values $p > 0,85$ (p-value $< 0,05$) in all binary comparisons.

Conclusions: Facial features of PMM2-CDG patients present a recognizable and differentiated gestalt. As far as this application is open access and easily available, it is considered a useful tool for daily clinical practice. Further studies will study these dysmorphic features in early ages and its correlation with neurological involvement and genotype.

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P06.60D
RNASeq profiling of Pompe disease patients reveals a compensatory transcriptional response centered around mTOR inhibition

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Pompe disease is a lysosomal storage disorder which is caused by alpha-glucosidase deficiency and leads to accumulation of glycogen in the lysosomes. The disease phenotype is mainly characterized by muscle weakness and hypotonia, finally resulting in respiratory difficulties. While the clinical manifestations of the disease have been well documented, it remains unclear what the consequences of the excessive glycogen storage are at the transcriptional level. For this purpose, we performed RNAseq on fibroblasts of four Pompe patients and compared this to four controls. Transcriptional profiling revealed the presence of a compensatory effect of the cells to increase lysosomal functionality which seems to be centered around inhibition of the mTOR (mammalian Target of Rapamycin) pathway. It has been shown that inhibition of the mTOR pathway leads to nuclear localization of TFEB and subsequent activation of the CLEAR (Coordinated Lysosomal Expression and Regulation) gene network. In all tested Pompe patients we observe a 6-10 fold transcriptional upregulation of the DEPTOR mRNA, which is an inhibitor of the mTOR pathway. Additionally, an mRNA downregulation of several mTOR activating genes including GAS6, Rhes and MC4R was detected as well, further hinting to an overall inhibition of the mTOR pathway. These results suggest that cells of Pompe patients initiate a compensatory response to try to restore the disturbed lysosomal functionality. Whether this cell intrinsic restoration mechanism is specific for Pompe patients or is common for lysosomal storage diseases in general remains to be investigated.

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P06.61A
A biallelic novel mutation in the COQ5 C-methyltransferase gene gives a diagnosis of a new sub-type of primary CoQ10 deficiency

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Primary Coenzyme Q10 (CoQ10; MIM# 607426) deficiencies are an emerging group of inherited Mitochondrial disorders with heterogeneous clinical phenotype. Over a dozen genes are involved in the biosynthesis of CoQ10, and mutations in several of these are associated with human disease. However, mutations in COQ5 (MIM# 616359), catalyzing the only C-methylation in the CoQ10 Synthetic pathway, have not been implicated in human disease. Here, we report three female siblings of Iraqi-Jewish descent, who had varying degrees of cerebellar ataxia, encephalopathy, seizures, and cognitive disability. We describe the remarkable mechanism of this molecular mutation and outline some of the major challenges in diagnosis via molecular analysis. In the cases described, a duplication in a non-coding region of the CoQ5 gene was not diagnosable by WES, and only through WGS with targeted focus of a suspicious area, was it possible to reach a diagnosis of a new disease with treatment potential. Duplications in the COQ5 gene, lead to reduced levels of CoQ10 in peripheral white blood cells of all affected individuals and reduced CoQ10 levels in the only muscle tissue available from one affected proband. CoQ10 supplementation led to clinical improvement and increased the concentrations of CoQ10 in blood. This is the first report of primary CoQ10 deficiency caused by loss of function of COQ5, with delineation of the clinical, laboratory, histological, and molecular features, and insights regarding targeted treatment with CoQ10 supplementation. Early diagnosis of this disease may be of great value for for successful treatment and intervention strategies.

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P06.62B

U-IMD: Unified European Registry for Inherited Metabolic Disorders as a patient database for MetabERN

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Introduction: Inherited metabolic disorders (IMD) are a prominent group of over 700 monogenic rare diseases with an estimated total birth frequency of at least 1:500. In 2017, the European Commission established the non-profit network MetabERN, which connects 69 health care providers in 18 EU countries and delivers care to approximately 43,000 patients with IMDs. An important instrument to improve diagnosis, treatment and wellbeing of patients is a systematic collection of data in a registry.

Project description: The Unified European Registry for Inherited Metabolic Disorders (U-IMD) project started in February 2018. The project has three major activities: a/to establish a patient registry for the MetabERN based on the common data elements of the European Platform on Rare Disease Registration; U-IMD will be the first unified European registry that encompasses all IMDs, b/to upgrade already existing IMD registries to the standard of U-IMD, starting with the registry of the International Working Group on Neurotransmitter Related Disorders (iNTD) and c/to develop a standard for minimal core data sets shared by the MetabERN and the European Rare Kidney Disease Reference Network (ERKNET). The diverse nature of the heterogeneous etiological and clinical spectrum of the IMDs necessitates collection of a minimal set of common data elements and the usage of controlled and standardized vocabularies such as Human Phenome Ontology or WHO ATC classifications for the description of the clinical phenotype and treatment strategies, respectively.

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P06.63C

RNA-seq of whole-blood in Asian extreme childhood obese subjects indicates defects in oxidative phosphorylation and mitochondrial pathways

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Introduction: Whole-blood transcriptomics have highlighted novel disease pathways. We aimed to evaluate for differentially expressed genes (DEGs) in whole-blood between 34 extreme obese and 30 normal weight children. We further evaluated the role of identified DEGs with diabetes related phenotypes in 138 independent childhood samples.

Methods: Extreme childhood obesity was defined as onset <10 years of age and BMI ≥98th percentile. Normal weight subjects were defined as a BMI between 18.5 kg/m^2 and 24.5 kg/m^2. Whole-blood was collected in Paxgene tubes. RNA were library-prepped using Illumina Truseq v2 kits and sequenced on the Hiseq4000 in 15-plexes. Sequenced reads were QCed using Illumina chastity filters and FastQC. QCed reads were mapped to HG19 using tophat and transformed to CPM and normalized between-samples using log2 transformation. All association tests were performed in Deseq2, adjusted for age, sex, ethnicity and sequencing batches.

Results: Mean reads of samples were 20.5 million reads. Analysis for DEGs between obese and normal weight children identified 34 DEGs (Bonferroni adjusted-p < 0.05 and fold-change >1.4 or <-1.4). DEGs were over-represented in oxidative phosphorylation and mitochondrial pathways (*COX7C*, *NDUFS5*, *NDUFS4* and *UQCRB*). We further evaluated the role of DEGs with multiple diabetes related traits in an additional 138 samples and identified nominally significant associations with fasting glucose, fasting insulin and HOMA-IR levels (p between 0.044 and 0.0006, *EMILIN2*, *HMGB2*, *NDUSF4*, *NDUSF5*, *PVRL2*, *STAB1* and *TXN*).

Conclusion: Whole-blood RNA-seq analyses identified genes in oxidative phosphorylation and mitochondrial pathways associated with extreme childhood obesity that may also play a role in diabetes-related pathways.

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P06.64D
Substrate reduction therapy approach for Sanfilippo C syndrome: use of iPSC and iPSC-derived neurons from patients as cellular models

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Sanfilippo C syndrome is a rare lysosomal storage disorder caused by mutations in the *HGSNAT* gene, which encodes an enzyme involved in heparan sulphate (HS) degradation. It is characterized by a severe and progressive neurodegeneration for which no effective treatment exists.

Previously, we demonstrated the usefulness of siRNAs targeting *EXTL2* genes (involved in HS synthesis) as an effective short-term substrate reduction therapy (SRT), on Sanfilippo C patients' fibroblasts. Now, we use different lentiviral vectors encoding shRNAs targeting *EXTL2* to analyse their long-term effect. We observe a clear reduction in *EXTL2* mRNA levels sixty days after transduction and an evident decrease of the HS amounts.

Due to the good results obtained, now we are using neurons derived from patients' induced pluripotent stem cells (iPSC) as a cellular model. We are using an established protocol to differentiate those iPSC into neurons within a week. Neurons show mature signatures and functional properties after one month. This technique will provide new insights in the usefulness of this treatment in the main affected cell type. To evaluate this therapeutic option, we are analysing the neurons, focusing on aspects such as the inhibition of the *EXTL2* gene at the mRNA level or the accumulation of HS over time by immunocytochemistry.

Our preliminary results in patients' fibroblasts indicate that shRNAs could be a long-term SRT and open a door for the development of a promising therapeutic approach for Sanfilippo C syndrome.

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P06.65A
Characterization of genetic variants in centenarians associated with obesity, metabolic syndrome and hypertriglyceridemia

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Introduction: It is likely that the supercentenarian's genome will not contain pathogenic variations or it's possible

to have some protective alleles. The aim of this study is to characterize genetic variations associated with obesity, metabolic syndrome and hypertriglyceridemia.

Materials and Methods: Our analysis was performed on a group of 17 supercentenarians and 34 controls. We used the publicly available whole-genome sequence database (Gierman HJ et al., 2014) and focused our attention on genetic variations at 25 genes associated with obesity and metabolic syndrome.

Results: We identified 5 variations in genes *TAS1R2, CLOCK, FABP2, ADRB2, TCF7L2* with pathogenic or protective effect, distributed unequally between the two studied groups. Two of the variations, in genes *CLOCK* and *FABP2*, can be discussed as polymorphisms of probably protective significance because their frequency is higher in the group of the supercentenarians. The variations in genes *ADRB2, TCF7L2* are with pathogenic significance and their frequency is again higher in the group of supercentenarians, which means that it is possible to refer to gene interactions that determine different penetrance or to impacts of other genetic mechanisms. According to some data, the *TAS1R2* gene polymorphism is risky for diabetes and dyslipidemia.

Discussion: The data for the higher frequencies of the possible protective variants in the *CLOCK* and *FABP2* and low frequencies of pathogenic variants in the *TAS1R2* and *ADRB2* genes in the group of supercentenarian compared to the control group is in line with our hypothesis but they need further research.

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P06.66B

Association study of candidate genes with diabetic nephropathy in North Indian Population

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Diabetic Nephropathy (DN) or Diabetic Kidney Disease (DKD) is characterized by functional and structural changes with the predominant changes in mesangial expansion, glomerular sclerosis and Glomerular Basement Membrane (GBM) thickening. It has been observed that approximately 20-30% of type 2 diabetes (T2D) patients are more likely to develop DN. Genetic vulnerability has been anticipated as an important factor for the development and progression of diabetic nephropathy and various research efforts have been executed worldwide to identify the susceptible genes for diabetic nephropathy. Several single nucleotide polymorphisms (SNPs) have been observed in different genes which have been found to play a major role in the genetic susceptibility to DN. In this study five SNPs, each from five candidate genes like *ELMO1, CD2AP, VEGFA, HLA-G* and *TERF1* have been evaluated to study the susceptibility of these genes in the development of DN. 1260 subjects (374 diabetic nephropathy samples and 886 healthy controls) have been included in this study. Genotyping was performed, using High throughput AGENA MassArray technique. Four SNPs, *ELMO1* (rs741301), *VEGFA* (rs2010963), *HLA-G* (rs1063320) and *TERF1* (rs2010441) showed strong association with the development of DN, whereas one marker rs1485780 (*CD2AP*) did not show any association. The study highlights that risk of diabetes-associated renal ailment may be enhanced by risk alleles at different susceptibility loci, in the presence of hyperglycemia. The grant to Gurvinder Singh by UGC-UPA Scheme and the grant No. F.8-2/2008(NS/PE),UGC,India to AJS Bhanwer through CPEPA has been acknowledged.

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P06.67C

Homozygous mutation p.Arg192Cys of the TALDO1 gene causes primary amenorrhea and hepatosplenomegaly without liver cirrhosis in an adult woman

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Introduction: Transladolase deficiency (TALDO, OMIM 606003) represents a recently recognized error of the pentose phosphate pathway. Until now, it has been reported in only 11 children, but there are no data about adult patients with this disorder

Material and Methods: We report about an 32-year-old Austrian woman with congenital hepatosplenomegaly, liver dysfunction, gonadal streaks and mild facial dysmorphia. She was born with neonatal oedema and atrial septum defect. In the neonatal period, she experienced sepsis with leucopenia and thrombocytopenia. At the age of one year, an inhomogenity of liver parenchyma was seen without the typical signs of cirrhosis. As the parents declined liver

biopsy at that time, it was performed in adulthood because of bleeding from oesophageal varices. Histology didn´t show features of cirrhosis and no portal hypertensia was diagnosed, however pulmonal-arterial hypertension was present. Cholelithiasis and pancytopenia persist until now, without any serious clinical problems. At the age of 32 years her liver is 6 cm, her spleen 12 cm large and the sex hormone status is comparable to that of a climacteric woman.

The diagnosis of TALDO was set using EXOM sequencing focusing primarily on hepatospenomegaly in the search for potential candidate genes.

Results: The homozygous mutation p.Arg192Cys of the *TALDO1* Gene, as found in our patient, was already reported in a 2 year old child with liver fibrosis and speech delay (Wamelink et al., 2008).

Conclusions: In summary, this is the first report ever about an adult woman with Transladolase deficiency describing her specific clinical course.

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P06.70B
Whole-exome sequencing of a case-unaffected parent's trio using barcoded DNA from dried blood spots

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Introduction: The National Hospital of the Faroe Islands has screened new-borns for heritable traits since 1986 using blood samples collected on filter paper. To use the current 22.000 filter cards for genetic research has retrospect advantages, however, challenging due to degraded samples and the limited quantities of DNA obtained from each card, which may be inadequate for next-generation sequencing (NGS) analyses. As NGS introduces it´s own limitations with short reads, we investigate the possibility to use dried blood spot (DBS) samples together with linked-reads for detection of a disease causing mutation (c.95A>G) in carnitine-transporter deficiency disease, and whether this approach may replace the trio information for true haplotype detection.

Materials and Methods: The case-unaffected parent's trio, was whole-exome sequenced using DNA obtained from DBS and whole blood (WB). DNA was barcoded using the Chromium™ Genome Kit. Exomes were captured using the SureSelectXT Human All Exon kit and sequenced on the NextSeq 500. The linked-reads were aligned to the reference genome (GRGh37/hg19) and variants were called using Freebayes.

Results: The c.95A>G variant was sequenced with a mean-coverage of 83 and 23 in the WB and DBS samples, respectively. Father and child were heterozygous (A/G) for the variant, while the mother was homozygous (A/A), which was consistent for DBS and WB.

Conclusion: DBS can be used for mutation identification when WB is unavailable. Linked-reads may be essential for detection of specific diplotypes when compound heterozygosity plays a role in the pathogenesis.Funding: The project is funded by the Danish and Faroese Governments.

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P06.71C
Next generation sequencing (NGS) for mitochondrial respiratory chain disorders (MRCD): new genes, cautionary tales and lessons learnt

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Introduction: MRCD diagnosis is an arduous journey, with early NGS being a logical diagnostic approach. We report our extensive analysis of trio whole genome sequencing (WGS) in 40 children with suspected MRCD, focusing on genes not previously associated with MRC function.

Methods: Trio WGS was performed at the Kinghorn Centre for Clinical Genomics using the Illumina HiSeq X sequencing platform: 95% of the nuclear genome covered to > 15× depth; mitochondrial genome covered to >3,000× depth. We identified SNVs and INDELS using GATK, copy number and structural variation using *ClinSV* and mitochondrial DNA variants using *mity*.

Results: Overall diagnostic yield was > 60% (most involving nuclear genes), including three putative new disease genes. Six cases with clinical, biochemical and/or enzymatic features consistent with a MCRD disorder had mutations in "non-MRCD" genes, including *ARX* (two

affected half-brothers), *SLC39A8* (two sibs with Leigh disease), and single cases with mutations in *HRAS* (Costello syndrome), *EPG5* (Vici syndrome), *SKIV2L* (Trichohepatoenteric syndrome) and *G6PC* (Glycogen Storage Disorder type 1a).

Conclusions: Suspected MRCD might be associated with "non-MRCD" genes, with potential explanations including: clinical picture is a phenocopy of MRCD (MRC abnormalities are a red herring); functional MRC abnormalities are real, indicative of a previously unrecognised contribution to the phenotype; the phenotype may be "blended" reflecting contributions from two disease genes. These findings highlight the importance of vigilance for "non-MRCD" genes as the genetic aetiology in certain cases, and the benefit of comprehensive, unbiased WGS. Research funded by a NSW OHMR Sydney Genomics Collaborative grant.

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P06.72D

Methylmalonic Aciduria cblB type cellular model: Hepatocyte differentiation from iPSC and pharmacological chaperones evaluation

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The understanding of the cellular and molecular mechanisms underlying inherited metabolic disorders (IMDs) is essential for developing new strategies for their prevention and treatment. Due to the genotype variability of IMDs and the upcoming of personalized medicine has prompted the emergence of developing new models. The aim of this work was the generation of a hepatic model of methylmalonic aciduria cblB type by hepatocyte differentiation of induced pluripotent stem cells (IPSCs) generated by reprogramming of patient-derived fibroblasts. This organic aciduria is caused by the deficiency of ATP: cob(I)alamin adenosyltransferase (ATR) encoded by the *MMAB* gene. Fibroblasts from a patient bearing a hypomorphic destabilizing mutation in this gene (p.Ile96Thr) were reprogrammed using a commercial kit based on Sendai virus vectors. After the molecular and functional characterization of the iPSC line, these cells were differentiated in vitro into definitive endoderm and then incubated with specific factors, aimed at hepatocyte differentiation. IPSC-derived hepatocytes expressed relevant hepatic markers analyzed by immunofluorescence. Finally, the hepatocytes generated were used for evaluation of potential pharmacological chaperones previously described (N-{[(4-chlorophenyl)carbamothioyl] amino]-2-phenylacetamide and 4-(4-(4-fluorophenyl)-5-methyl-1H-pyrazol-3-yl)benzene-1,3-diol)) in combination with hydroxocobalamin, providing evidences of its positive effect on the activity of the mutant ATR hepatocytes. Hence, our findings provide an experimental suitable model for the investigation of the hepatotoxicity of new drugs and the pathogenesis of this severe disease serving also as ex vivo platform for organoids generation and therapeutic applications.

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P06.73A

Optimization process of potential pharmacological chaperone development: Looking for a PMM2-CDG therapy

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The functional characterization of Phosphomannomutase 2 (PMM2) disease-causing mutations has suggested that PMM2-CDG could be a conformational disease. Therapies to ameliorate clinical symptoms could be addressed improving the protein folding to restore total or partially its native state. In this sense, from a 10,000 compound library screening the compound 1-(3-chlorophenyl)-3-3-bis(pyridine-2-yl)urea (compound VIII) stood out, based on its pharmacochemical properties, enhancing the enzymatic activity and stability of a number of destabilizing PMM2 mutations. These results provided a promising chemical structure as a starting lead for new therapeutic agents against this severe orphan disease. The aim of this work was setting-up an optimization process by methodological sequential rounds from a battery of chemical analogs of compound VIII in order to improve the physicochemical properties and cytotoxicity: Up to 795 analogs were evaluated and results showed 165 analogs that passed every reactivity filter by in silico SmartsFilter analysis. In a first selection of 25 analogs for in vitro analysis, 4 of these

25 structures have shown no concentration-dependent inhibitory effect on enzymatic activity, a mild stability improvement and higher PMM2 activity in a mutant cellular model bearing a destabilizing mutation (p.T237M). This workflow for developing a potential therapy for PMM2-CDG has shown a dynamic progression from a promising pharmacological chaperone to 4 new compounds that passed critical selection points, allowing the process to move forward to the next screening levels in another cellular models which will provide new potential structures with pharmacological effects.

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P06.74B

De novo heterozygous *HSPD1* variants: A novel mechanism in hypomyelinating leukodystrophy type 4?

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Hypomyelinating leukodystrophy type 4 (HLD4) (OMIM 612233) is a progressive neurodegenerative disorder characterized by hypotonia, psychomotor delay, acquired microcephaly, mental retardation, and seizures. Typically, the condition onsets in infancy and is fatal within the first two decades. Brain MRI is consistent with diffuse hypomyelination. Some patients have increased urinary ethylmalonic acid. HLD4 is attributed to autosomal recessive mutations in the *HSPD1* gene, encoding the mitochondrial Hsp60 chaperonin.

We present a 4 year-old boy with speech and motor delays. Brain MRI showed diffuse hypomyelination of the cerebral and cerebellar white matter. Examination revealed a normal head circumference, brisk reflexes, mild ataxia and bilateral postural tremor. Urine metabolic screening detected increased ethylmalonic acid. Whole exome sequencing was completed, and detected a de novo heterozygous *HSPD1* variant (c.139T>G, p.L47V).

Mass spectrometry analyses indicated similar amounts of mutant and wild type proteins in our patient's fibroblasts. We used an *Escherichia coli* genetic assay system to assess the function of the mutant Hsp60(L47V) protein. Previously, we showed by complementation that *E. coli* cells expressing the wild-type human Hsp60 protein and its co-chaperone Hsp10 protein can survive deletion of the otherwise essential and homologous *groESgroEL* genes of *E. coli*. Here, we found that co-expression of the wild-type human Hsp10 and mutant Hsp60(L47V) does not support growth of these *E. coli* cells. Our results demonstrate that the function of the mutant Hsp60(L47V) protein is compromised, at least when expressed in *E. coli*. This case highlights a possible novel autosomal dominant mechanism for HLD4.

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P07 Immunology and hematopoietic system

P07.01A

Association study of *TNFAIP3* gene polymorphisms in three different autoimmune diseases

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Introduction: Autoimmune diseases (AIDs) are complex diseases that share several susceptibility genetic loci. Tumor necrosis factor alpha inducible protein 3 (*TNFAIP3*) encodes the ubiquitin-modifying enzyme A20, that down-regulates inflammation by restricting NF-kB, a transcription factor that regulates expression of various pro-inflammatory genes. Variants in *TNFAIP3* gene have been described as associated with susceptibility to several AIDs. Here, we analyzed two *TNFAIP3* polymorphisms in Italian patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and Sjögren's syndrome (pSS), to verify if *TNFAIP3* is involved in genetic predisposition to AIDs also in Italian population.

Methods: We recruited 315 SLE patients, 196 pSS patients, 187 RA patients, and 236 healthy controls. Genotyping of rs2230926 and rs6920220 in *TNFAIP3* gene was performed by allelic discrimination assay. We carried

out a case/control association study and a genotype/phenotype correlation analysis.

Results: Higher risk to develop SLE was observed for rs2230926 (P=0.02, OR=1.92). No association was observed with the pSS susceptibility, but the variant allele seems to confer a higher risk to develop lymphoma in pSS patients. In RA patients, the presence of RF and ACPA resulted significantly associated with rs2230926 variant allele. We observed a significant association between the variant allele of rs6920220 and SLE (P=0.03, OR=1.53), pSS (P=0.02, OR=1.69) and RA (P=0.00001, OR=2.58) susceptibility. Furthermore, SLE patients carrying the rs6920220 variant allele showed a higher risk to develop pericarditis, pleurisy and kidney complications.

Conclusion: Our results support the importance of the *TNFAIP3* gene variants role in the development of different autoimmune diseases.

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P07.02B

Application of clinical exome sequencing panel in early onset autoinflammatory disease patients

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Introduction: Autoinflammatory diseases are usually difficult to diagnose due to their high phenotypic heterogeneity and variable expression. In some cases, diagnosis difficulties can be resolved using genetic testing. Next-generation sequencing (NGS) is a cost-effective approach to identify likely causative genetic variants. This identification can lead to a better understanding of the disease and increase the number of diagnosed patients. According to this, the main goal of this study was to use NGS to detect genetic variants likely to be causative of the disease in pediatric patients with autoinflammatory symptoms.

Materials and Methods: We performed target sequencing (TruSight™ One panel) in 26 samples from patients with clinical suspicion of autoinflammatory disease. Next, we performed bioinformatics analysis to detect likely causative genetic variants. For the most relevant candidates we corroborate our findings using Sanger sequencing.

Results: We evaluated the performance of the kit to capture autoinflammatory candidate genes. We found likely causative genetic variants of the disease in 10 patients (38.48%). This suggests a strong relationship between the genetic alteration and the observed phenotype. However, further studies of the segregation pattern of the variant are needed, as well as functional validation.

Conclusion: Target sequencing is a suitable approach to detect likely causative genetic variants in autoinflammatory disease patients. The use of NGS techniques in clinical immunology will lead to several benefits in basic science and clinical practice.

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P07.03C

Investigation of Peripheral Blood Mononuclear Cells (PBMC) Proteome Profile in Behcet's Disease

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Normal 0 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"-Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria; mso-ascii-font-family:Cambria; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Cambria; mso-hansi-theme-font:minor-latin;}

Introduction: Behçet's disease (BD) is a systemic inflammatory disorder. Investigation of the proteome profile will facilitate our understanding of disease processes. We aimed to identify proteins specific to BD and related pathways through proteomic analyses performed on PBMC samples.

Methods: Study groups were composed of active BD (N=33), inactive BD(N=26), and healthy controls(N=28). PBMC protein samples from each group were pooled and then separated using 2D-DIGE. Protein spots with at least 2 times differentially-expressed were compared among groups, and identified by MALDI-TOF-MS. Bioinformatic pathway analyses were carried out through KEGG, PANTHER and STRING databases.

Results: A total of 369 protein spots were detected by 2D-DIGE. 115 for active vs inactive BD, 118 for active BD vs healthy controls, 129 spots for inactive BD vs healthy

controls were identified. The strongest 45 spots were further analyzed through MALDI-TOF-MS. Fructose-bisphosphate aldolase-C, calreticulin, ficolin-1, fibrinogen alpha chain, fibrinogen beta chain, filamin-A, FUSE-binding protein-1, phosphoglycerate kinase-1, stathmin, vinculin, hnRNP-M, WD repeat-containing protein-1, HSPA8, myosin light polypeptide-6, talin-1 and tropomyosin alpha-3 chain were differentially expressed between groups.

Conclusion: We identified proteins that involve in glycolysis, complement/coagulation and coagulation activation pathways. The expression of proteins involved in ER protein processing (calreticulin, HSPA8, GRP78-BiP) were down-regulated in BD patients compared to healthy controls, which raised the importance of ER stress in Behcet disease pathogenesis. <!--EndFragment-->

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P07.04D

The human-restricted duplicated form of the α7 nicotinic receptor, CHRFAM7A: expression and transcriptional regulation in inflammatory cells

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Introduction: The α7 nicotinic acetylcholine receptor (CHRNA7) plays a role in the modulation of the inflammatory response through the activation of the "cholinergic anti-inflammatory pathway". In humans, a recombination event involving the exon 5 to 10 of *CHRNA7* gene, fused to four novel exons A, B, C and D (*FAM7A*), gave rise to the *CHRFAM7A* gene. This hybrid gene, located on chromosome 15q13-q14, 1.6 Mb apart from *CHRNA7*, is highly expressed in inflammatory cells, where it can regulate the anti-inflammatory effects of α7 activation. Acute treatment of macrophages with LPS down-regulates *CHRFAM7A* by a mechanism driven by NF-κB, paralleled by *CHRNA7* up-regulation. As studies are emerging, which identify *CHRFAM7A* expression alteration in inflammatory or infective pathologies, the regulation of its expression may become a key step in the modulation of inflammation. However, the region driving the transcriptional regulation of *CHRFAM7A* gene in human immune tissues is largely unknown.

Materials and Methods: human macrophages and THP-1 cell line have been used to characterized the *CHRFAM7A* regulatory region.

Results and Conclusions: we provide a detailed analysis of the *CHRFAM7A* gene regulatory region and its pro-inflammatory stimuli responsiveness. Furthermore, given the anti-inflammatory potential of the acetylcholinesterase inhibitor donepezil, we investigated the *CHRFAM7A* expression profile in macrophages treated with donepezil, showing an unexpected up-regulation of both *CHRFAM7A* and *CHRNA7* gene, thus highlighting a possible role for *CHRFAM7A* gene product in the control and modulation of the cholinergic anti-inflammatory pathway, and/or in the modulation of CHRNA7 function.

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P07.05

AmiRNA-146a and miRNA-155-5p in CML patients before and after initiation of TKI therapy

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Introduction: Tyrosine kinase inhibitors (TKIs) are the first-line therapy for most chronic myeloid leukemia (CML) patients, however some are unresponsive to it or develop resistance. Recently, microRNAs (miRNAs) have been implicated in the progression of CML and the development of TKI resistance. miRNA-146a and miRNA-155-5p are involved in MAPK signaling pathway, cell proliferation and apoptosis. In this study we aimed to investigate expression of miR-146a and miR-155-5p in CML patients and, where possible, to identify how TKI treatment affects this expression.

Materials and Methods: Bone marrow (BM) samples were obtained from newly diagnosed CML patients (n = 16) and healthy controls (n = 18). From one patient we took 3 samples: one before TKI therapy initiation and two samples after (6, 12 months). Quantitative assessment of the expression of miRNA-146a and miRNA-155-5p was performed by qRT-PCR using qScript™ microRNA cDNA Synthesis Kit, specific primers, iQ™ SYBR® Green

Supermix and Bio-Rad CFX96 Real-Time PCR Detection System.

Results: miRNA-146a expression was significantly elevated in CML patients (p = 0,006), expression of miRNA-155-5p did not differ between groups. Both tested miRNAs showed constant decrease in expression in 6 and 12 month after TKI therapy initiation.

Conclusions: miRNAs, including miRNA-146a, miRNA-155-5p, are promising biomarker candidates for CML diagnosis and prognosis. The evident drop in expression levels of these miRNAs after TKI therapy suggests that they might also be viable targets for monitoring drug response. However, the precise contribution of miRNA-146a and miRNA-155-5p to CML pathogenesis remains to be further elucidated.

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P07.06B

Whole Genome Sequencing in a cohort of children with hypereosinophilia driven autoimmunity

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Hypereosinophillia is a common, often transient, phenotype with a broad differential. However, there exists a small subset of patients that have a constitutively increased eosinophil count, resulting in severe end organ autoimmunity and requiring ongoing immunosuppressive therapy. Previously described genetic causes for these rarer forms of hypereosinophilia include; chromosomal rearrangement promoting bone marrow eosinophil production, TCR clonality or recently described single gene disorders (e.g. STAT3 and MALT1). We present a cohort of three children who presented with dysmorphic features and hypereosinophilia driven autoimmunity of the skin and gastrointestinal system. These children all had extensive genetic testing including; microarray, TCR clonality studies, chromosomal rearrangement FISH studies and exome sequencing, however, only variants of uncertain significance were identified. We therefore performed whole genome sequencing (WGS) with the hypothesis that an intronic regulatory variant is responsible for the hypereosinophilia driven autoimmunity observed. We present here a detailed phenotyping of this cohort, the variants of uncertain significance that were identified by microarray and exome sequencing and the results of the WGS. We have identified several previously undescribed candidate variants that could explain the hyperosinophilia driven autoimmunity phenotype. We have also performed immunological assays to validate these variants. We conclude that WGS is the best investigation in rare cases of hypereosinophilia driven autoimmunity. This test improves time to diagnosis, helps rule out known chromosomal/single gene causes and identifies novel intronic variants that are overlooked by exome testing alone.

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P07.08D

Loss of the phosphatase *PTPRJ* causes migration defects of megakaryocytes and thrombocytopenia

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Inherited thrombocytopenias (IT) are characterized by decreased circulating platelets that can be associated with other phenotypes (bone marrow aplasia, haematological tumors, renal failure).

Through exome sequencing we identified a new recessive IT due to loss of *PTPRJ* (Protein Tyr-Phosphatase, Receptor type J).

In two affected siblings we observed two heterozygous variants causing a premature stop insertion on both alleles and the loss of mRNA and protein, as we observed in patients' platelets. PTPRJ is a transmembrane tyrosine-phosphatase highly expressed in megakaryocytes (Mks) and platelets. A mouse model of PTPRJ inactivation shows defects in Mk migration, platelet production, platelet activation and aggregation. The two probands with *PTPRJ* mutations presented with thrombocytopenia and moderate

spontaneous bleeding. Patients' platelets showed defective activation and aggregation and a global decrease in tyrosine phosphorylation after stimulation with a GPVI agonist associated with reduced activation of the tyrosine kinase Src. Mks differentiated *in vitro* from patients' blood progenitors showed impaired maturation and defective migration. Exploiting a zebrafish line with fluorescently labeled thrombocytes, we demonstrated a significant decrease of the circulating thrombocytes in the PTRPJ KO with morpholino and the phenotype rescue through injection with human wt mRNA. Moreover, silencing of *PTPRJ* in the human Mk cell line Dami cells induced the migration and maturation defects observed in patients' Mks.

In summary, we discovered a novel form of IT. Pathogenetic mechanisms include impaired Mk migration and maturation. These abnormalities may be mediated by reduced activation of Src, which is therefore recognized as a target of *PTPRJ* in humans.

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P07.09A

The multiple myeloma risk allele at 5q15 lowers *ELL2* expression and increases ribosomal gene expression in malignant plasma cells

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Multiple myeloma (MM) is the second most common hematologic malignancy that forms in plasma cell. Recently, we identified *ELL2* as a susceptibility gene for MM. To understand its mechanism of action, we performed expression quantitative trait locus (eQTL) analysis in $CD138^+$ plasma cells from 1,630 MM patients from four populations. We show that the MM risk allele lowers *ELL2* expression in these cells ($P_{combined}=2.5\times10^{-27}$; $\beta_{combined}=-0.24$ s.d.), but not in peripheral blood or other tissues. A total of 67 single-nucleotide polymorphisms and 5 small insertions/deletions are highly correlated with the best-supported sentinel MM risk variant (rs1423269) and the strongest *ELL2* expression variant (rs9314162) ($r^2 > 0.8$). Using bioinformatic approaches we identified 8 variants that might alter the efficiency of *ELL2* transcription. We made luciferase vectors for each of these variants and transfected them into three MM plasma cell lines (L363, OPM2, and RPMI-8226) and two cell lines representing other hematologic lineages (K562 and MOLM-13). Among those, three risk variants (rs3777189-C, rs3777185-C and rs4563648-G) yielded decreased luciferase activity relative to their corresponding protective variants in plasma cell lines, but not in non-plasma cell lines. Further analysis reveals that the MM risk allele associates with upregulation of gene sets related to ribosome biogenesis, and knockout/knockdown and rescue experiments in plasmocytoma cell lines support a cause-effect relationship. Our results provide mechanistic insight into MM predisposition.

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P07.10B

Genomic and functional evaluation of the role of *TNFSF14* gene in susceptibility to multiple sclerosis

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Over 200 multiple sclerosis (MS) susceptibility genes were identified. Among these, the strongest non-HLA signal in the Italian population maps in the Tumor Necrosis Factor (ligand) superfamily member 14 (*TNFSF14*) gene encoding for LIGHT, a transmembrane glycoprotein expressed on various immune cells and involved in dendritic cells (DC) maturation. We demonstrated through a fine-mapping approach that an intronic variant is the primarily associated one. Cis-eQTL analysis from different databases showed that carriers of MS risk allele have a lower *TNFSF14* RNA expression in EBV-transformed lymphoblastoid cell lines (Geuvadis, Bioportal, Gtex) and in PBMCs (Gtex). These data are consistent with the imbalance against the risk allele observed in heterozygous individuals ($p < 0.0001$, RNAseq on 97 lymphoblastoid cells, Geuvadis). Consistently, in PBMC of 84 Italian MS and 80 healthy controls (HC), individuals with MS risk genotype produced lower levels of *TNFSF14* transcript ($p = 1.1e\text{-}4$) and MS patients were the minor producers ($p = 0.031$). Analysis on peripheral blood of HC(N=37) with flow cytometry showed that in myeloid DC (CD11c+) the homozygous individuals for the risk allele had a higher percentage of LIGHT positive cells (p-value = 0.04). In conclusion, we propose that an altered *TNFSF14* expression in immune cells driven by an intronic variant can contribute to MS pathogenesis. Particularly, this variant seems to be associated with a low *TNFSF14* RNA expression in a mixed population of PBMCs and with a higher percentage of *LIGHT* positive cells in myeloid dendritic cells, suggesting a cell specific influence of this variant on *LIGHT* expression at the protein level.

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P07.11C

Single-cell transcriptomics uncovers cellular and molecular determinants of tissue myeloid cell heterogeneity in homeostasis and cancer

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Introduction: The innate immune system is highly complex and comprises cell populations with organ-specific properties. Key open questions include how the tissue of origin shapes the phenotype of myeloid cells and how this heterogeneity is altered in pathological conditions. Here, we combine single-cell (sc)RNA-Seq and immunophenotypic analyses to build a comprehensive view of the tissue mouse myeloid cell landscape, at steady state and in models of pancreatic adenocarcinoma (PDAC).

Methods: Tissue-resident myeloid cells ($CD45^+CD11b^+$) were isolated from 9 tissues in healthy mice (blood, bone marrow (BM), spleen, lung, liver, pancreas, brain, colon and intestine) and subjected to scRNA-Seq. Ortho- or hetero-topic PDAC models were established by intrapancreatic or subcute injection of DT6606 cells in C57/BL6 mice. Tumor-infiltrating, circulating and BM myeloid cells were analysed throughout disease progression by scRNA-Seq, immunophenotypic and histological analyses.

Results: scRNA-Seq analysis shows tissue-specific heterogeneity in monocytes, neutrophils, dendritic cells and macrophages populations at the steady state and identified putative gene networks underlying this organ specialization. Pancreatic cancer had a dramatic impact on the composition and transcriptional heterogeneity of tumor-infiltrating and circulating pools of monocytes and neutrophils, and also affected BM myelopoiesis at the single-cell level. Differential gene expression analysis in tumor-associated vs. steady-state myeloid cells revealed transcriptional programs aberrantly activated in the PDAC immune microenvironment.

Conclusions: Our analyses link cellular and molecular alterations in the immune microenvironment to the progression of PDAC. These results have implications for the design of cell and gene therapy strategies aiming at stimulating anti-tumor immunity in pancreatic cancer.

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P07.12D

A new workflow for classification of genetic variants pathogenicity applied to hereditary recurrent fevers by the International Study Group for Systemic AutoInflammatory Diseases (INSAID)

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Background: Hereditary recurrent fevers (HRF) are rare inflammatory diseases sharing similar clinical symptoms, and effectively treated with anti-inflammatory biological drugs. Accurate diagnosis of HRF relies heavily on genetic testing, yet in the big data era the clinical significance of most gene variants remains unsolved or controversial.

Methods: We configured a MOLGENIS web-platform to share and analyze pathogenicity classifications of the variants, and to manage a consensus-based classification process. Four experts in HRF genetics submitted independent classifications of 858 variants of 4 well known HRF genes: *MEFV, TNFRSF1A, NLRP3* and *MVK*. Classifications were driven to consensus by recruiting 4 more expert opinions and by targeting discordant classifications in 5 iterative rounds.

Results: A consensus classification was reached for 804/858 variants (94%). None of the unsolved variants (6%) remained with opposite classifications (e.g. pathogenic versus benign). New mutational hotspots were found in all genes. We noted a lower pathogenic variant load and a higher fraction of variants with unknown or unsolved clinical significance in the *MEFV* gene.

Conclusion: Applying a consensus driven process on the pathogenicity assessment of experts yielded rapid classification of almost all variants of four HRF genes. The high-throughput database will profoundly assist clinicians and geneticists in the diagnosis of HRFs. The configured MOLGENIS platform and consensus evolution protocol are usable for assembly of other variant-pathogenicity databases. The MOLGENIS software is available for reuse at http://github.com/molgenis/molgenis, and the specific HRF configuration is available at http://molgenis.org/said/. The HRF pathogenicity-classifications will be publically on the INFEVERS database at http://fmf.igh.cnrs.fr/ISSAID/infevers/.

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P07.13A

Atypical paroxysmal nocturnal hemoglobinuria presenting with autoinflammatory symptoms is caused by germline and somatic mutations involving PIGT

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disorder of the blood-forming system. Typically, affected hematopoetic stem cells (HSCs) in PNH harbour a single somatic loss-of-function mutation in the X-linked PIGA gene. Herein we report four cases of this new subgroup: A predisposing germline mutation in PIGT, which is an autosomal gene of the glycosylphosphatidylinositol (GPI)-

anchor synthesis pathway, is followed by a second somatic hit. Deep sequencing and array-CGH identified acquired deletions on chromosome 20q in PNH cells that include PIGT and a commonly deleted region in myelodysplastic syndromes, that is known to be differentially methylated. This results in a complete loss of expression of certain genes at this locus which is also thought to contribute to the clonal expansion. The deficiency of GPI-anchored proteins on PNH cells results in a lack of complement regulation.In contrast to classical PNH, PIGT mutations impair loading of substrate to the anchor and thus result in an accumulation of unbound GPI molecules.This difference in the pathophysiology can also be visualised by FACS analysis of blood: While CD55 and CD59 expression is reduced in all PNH cells, the atypical PNH cells can be discriminated by a specific antibody that binds free GPI anchors. Besides classical PNH symptoms of anemia, thrombosis, and hemolysis, patients with PIGT-mutations also manifest with additional autoinflammatory symptoms. It is hypothesized that the free GPI-anchor that accumulates in affected cells is causally related to autoinflammation. Based on these findings, we propose the new entity of atypical PNH.

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P07.14B
Functional study of Peptidylarginine Deiminase genes in arthritis model mice

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Previously, peptidylarginine deiminase type 4 (PADI4) was identified as a susceptibility gene for Rheumatoid arthritis (RA) by genome-wide association studies. Peptidyl citrulline is a target antigen of anti-citrullinated peptide antibodies (ACPAs), and only PADs (translated protein from PADI genes) can provide peptidyl citrulline via modification of protein substrates. Also the distribution of PADI4 and PADI2 has overlap in immune cells. The aim of this study was to investigate the relationship between PADI4 gene and PADI2 gene in the progression of RA.

Padi4−/− DBA1J and wild-type mice were immunized with bovine type II collagen (CII) to develop collagen-induced arthritis (CIA). Expression of various inflammatory cytokines and Padi genes in immune cells was detected by real-time TaqMan assay. Cytokine concentration in sera was measured by enzyme-linked immunosorbent assay. Localization of PAD4 and PAD2 protein was indicated by immunohistochemistry. We also generate Padi2−/− mice and performed experimental arthritis. We demonstrated that the clinical disease score was significantly decreased in Padi4−/− mice and Padi4 expression was induced by CII immunization. In Padi4−/− mice sera, serum anti-type II collagen (CII) IgM, IgG, and inflammatory cytokine levels were also significantly decreased compared with those in wild-type mice sera. Interestingly, Padi2 expression was compensationally induced in CD11b+ cells of Padi4-/- mice. We also demonstrated that the clinical disease score was significantly decreased in Padi2−/− CIA mice.

It appears that Padi4 and Padi2 enhance collagen-initiated inflammatory responses. Our results revealed that PAD4 affected on expression of various cytokines and also controlled Padi genes.

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P07.15C
Rare regulatory variant in the MEF2D gene is associated with SLE in Swedish patients and contributes to the gene regulation and splicing

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Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disorder with heterogeneous clinical manifestations and complex etiology. The common associated SNPs explain only a small part of disease heritability suggesting the contribution from rare genetic variants, undetectable in GWAS. We searched for novel rare variants associated with SLE.

Materials and Methods: 144 SLE patients and 17 controls were used for targeted re-sequencing of coding and conserved regulatory regions within and around 215 candidate genes. The variant enriched in cases was validated by genotyping in additional 360 patients and 822 healthy controls. Fisher's exact test and logistic regression were used for genetic association analysis. The regulatory effect of the novel variant was studied by EMSA, luciferase reporter assays and minigenes.

Results: We identified a novel rare regulatory variant rs200395694 located in the *MEF2D* gene encoding for the myocyte-specific enhancer factor 2D transcription factor associated with SLE in Swedish patients (total 504 SLE patients and 839 healthy controls, $p = 0.013$, CI=1.1-10). The risk allele was strongly associated with the triad of disease manifestations including Raynaud's phenomenon, anti-RNP and anti-Sm antibodies ($p = 0.00046$, CI 5.05-∞). The region has properties of an active cell-specific enhancer, differentially affected by the alleles of rs200395694. In addition, the risk allele exerts inhibitory effect on the splicing of the alternative tissue-specific isoform, and thus may modify the target gene set regulated by this isoform.

Conclusions: We present evidence of genetic association of a novel rare regulatory variant rs200395694 with SLE in Swedish patients.

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P07.16D
A novel germline mutation in *GP1BA* gene in family with hereditary macrothrombocytopenia

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Introduction: Hereditary thrombocytopenias are a rare and heterogeneous group of disorders, associated with approximately 30 causal genes involved in the process of megakaryopoesis and thrombopoesis. Pathological mutations lead to disruption of these processes and origin of thrombocytopenia.

Materials and Methods: We identified a family with autosomal dominant thrombocytopenia and increased platelet volume. We performed analysis of eight family members: four of them showing signs of thrombocytopenia, two healthy family members and one family member with borderline platelet count. Exome libraries were prepared according to the protocol for Nimblegen SeqCap EZ Exome v3 and sequencing was performed on NextSeq 500 for all of them. Found variants of individuals with thrombocytopenia phenotype were compared to variants of healthy family members.

Results: Whole-exome sequencing identified a heterozygous single-nucleotide change in *GP1BA* (exone2: c.176T>G), encoding a p.Leu59Arg substitution in the N-terminal domain, segregating with macrothrombocytopenia. This is until recently undescribed variant. In this study, we also analysed structural effect of found sequence variant *in silico*. In particular, we used crystal structure of N-terminal domain of human platelet receptor GPIb-alpha. Replacement of aliphatic amino-acid Leu 59 with charged, polar and larger arginine most probably disrupts the protein structure.

Conclusions: A germinal GP1BA mutation (exone2: c.176T>G) disrupts the molecular structure of the protein and is the reason of hereditary thrombocytopenia in this family.

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P07.17A
A novel susceptibility locus *CD53* in tuberculosis identified by pathogen lineage-based genome-wide association study

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Tuberculosis (TB) is a major global infectious disease that is caused by *Mycobacterium tuberculosis* (*M. tb*) and TB onset is known to be affected by host genetic factors. In this study, we focused on the heterogeneity of *M. tb* lineages and assessed its possible interaction with host genetic factors. Genome-wide association analyses stratified by pathogen lineage information and age at onset revealed that two SNPs on chromosome 1p13 were specifically associated with non-Beijing lineage infected old age onset cases

(p = 4.86E-08, OR=1.72 [95%CI=1.41-2.09], n = 314), but were not associated with Beijing lineage infected old age onset cases (p = 0.0870, OR=1.26 [95%CI=0.97-1.64], n = 155), when we compared them to the population matched 782 healthy controls. These SNPs were associated with both East-African Indian (EAI) and Euro-American lineages in the non-Beijing lineage group. These SNPs were located near *CD53*, which encodes a leukocyte surface glycoprotein and has not been reported to be associated with TB onset. However, interestingly, one of the significant SNPs was previously reported as a cis-expression quantitative trait locus (eQTL) of *CD53* expression level in dendritic cells infected by *M. tb*. This is the first report of TB pathogen lineage-based genome-wide association study and successfully identified a TB-associated locus at a genome-wide significance level. The present results indicated that host genetic risk in TB is affected by pathogen genetic background and demonstrate the importance of analyzing the interaction between host and pathogen genomic variations.

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P07.18B

Non-response to vaccines: still an enigma? B-cell transcription factor POU2F2/OCT2 is a potential candidate

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Unresponsiveness to vaccines affects 2-10% of individuals, representing an extraordinary limitation for infection prevention worldwide. Genetic determinants are still mainly unknown, although in recent years GWAS identified potential susceptibility loci (HLA-DQ, HLA-DR, CXCR5). We investigated a patient and her daughter with unresponsiveness to vaccines (tetanus, diphtheria, hepatitis B, poliovirus) and intermittent infectious episodes, but otherwise unremarkable clinical history. Lymphocyte proliferation assay to tetanus and diphtheria toxoids was highly impaired, with selective deficit of B-memory cells and IgM production. Whole-exome sequencing revealed a shared heterozygous frameshift variant (c.1285dupC;p.Leu429-ProfsTer73) affecting *POU2F2* (19q13.2), a non-OMIM gene with low tolerance to loss-of-function variations (pLI=0.97), encoding the transcription factor OCT2 (octamer-binding protein 2) that regulates immunoglobulin expression in germinal center B-cells. The variant was unreported in gnomAD and was shown to segregate in the family and to have occured *de novo* in the mother. Importantly, heterozygous knock-out mice show a pathological phenotype restricted to immune/hematopoietic system with reduction of B-cells and IgM, thus recapitulating our patients' phenotype. Analysis of mRNA from B-LCLs and fibroblasts of both carriers revealed a stable mutant trascript and excluded mRNA decay, suggesting a dominant-negative effect; in contrast, somatic *POU2F2* amplifications, leading to demonstrated overexpression, have been described in diffuse large B-cell lymphomas. Further functional assays showed severe deficiency of switched memory B-cells and reduced surface and intracellular immunoglobulin expression in both patients. In conclusion, our preliminary findings identified a novel gene likely involved in B-cell anergy and highlighted POU2F2 as an attractive target for enhancing humoral immune response to vaccination.

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P08 Intellectual Disability

P08.01A

Targeted NGS of the TSC1/TSC2 genes

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Tuberous sclerosis TSC (MIN:191100,613254) is an autosomal dominant disorder characterized by benign tumor growths in multiple organ systems. In 75-90% of cases TSC is due to mutations in the TSC1 (OMIM # 605284) or TSC2 (OMIM# 191092) genes. Somatic mosaicism potentially account for up to 26% of TSC cases. We report the results of NGS analysis in 3 familial and 8 sporadic unrelated cases referred with clinically diagnosed (9) or highly suspected TSC (2). DNA samples from peripheral blood leukocytes

and oral mucosa were analyzed by AmpliSeq custom panel covering the coding sequence of the two genes. NGS was performed on the Ion Torrent PGM platform. Data analysis was performed with ION Torrent Suite v.5; median read depth was ≥ 500x. Variants were annotated with wANNOVAR and filtered based on MAF (<1%), phylogenetic conservation and CADD score; pathogenicity was evaluated by 12 *in silico* tools and Sanger sequencing validation performed for all the filtered likely pathogenic variants. We identified 6 heterozygous seemingly germ-line mutations, two of which novel, in cases with a definite clinical diagnosis: 2 *TSC2* frameshift deletions (NM_000548: *c.5076delG;c.935delT*) and1 *TSC2* splice-mutation *(*NM_000548:*c.2221-2A>C)*, 2 *TSC1* rare single nucleotide variants (NM_000368:*c.569 C>G;c.647 T>C)* and 1 frameshift deletion (NM_000368; *c.709_716 del)*. The latter was present in the DNA of the proband's father as mosaic mutation at about 10% of mutant allele frequency. Five subjects remained without a molecular diagnosis; we plan to extend our NGS analysis in negative samples with the inclusion of TSC1 and TSC2 promoter, UTRs and intronic regions

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P08.02B

Microduplication of the 13q31.3 miR17-92 cluster results in a syndrome with features opposite to those associated with Feingold syndrome 2

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Deletion of the miR17~92 cluster is associated with Feingold syndrome type 2 (OMIM: #614326) characterized by short stature, microcephaly, skeletal abnormalities, and intellectual disability.

In the present study, we report a female individual presenting with opposite features such as tall stature and macrocephaly, besides developmental delay, skeletal and digital abnormalities and a *de novo* ~840kb duplication of 13q31.3 (91166748-92010901) encompassing only miR17~92 cluster. Two individuals carrying overlapping duplications of the miR17~92 cluster including the proximal *GPC5* were previously described. They similarly present with macrocephaly, developmental delay, skeletal and digital abnormalities as well as growth abnormalities. These latter two cases and published features of individuals with even larger 13q31.3 overlapping duplications suggested that gene expression imbalance of *GPC5* could be causative. The limited extent of the rearrangement and the normal expression level of *GPC5* in cells of our proband, however, provides evidence against this hypothesis.

Our results suggest that duplication of the miR-17~92 cluster is linked to a new syndrome characterized by features mirroring those of Feingold syndrome type 2, which is associated with haploinsufficiency of the region. Whereas deletion of the region is linked with short stature and microcephaly, duplication is on the contrary associated with overgrowth and macrocephaly. Similar dosage dependent mirror phenotypes on BMI and head circumference have been previously reported for deletion and duplications of the 1q21.1, 2p15, 16p11.2 BP4-BP5, 16p11.2 BP2-BP3 and 17p11.2 CNVs.

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P08.03C

15q13.3 microdeletion and microduplication in patients with neurodevelopment disorders

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The proximal long arm of chromosome 15 contains a cluster of low copy repeats (LCRs), located at breakpoints BP1-BP5. These mediate various deletions and duplications via non-allelic homologous recombination. BP4-BP5 microdeletion/duplication syndrome may include features of ASD, a variety of neuropsychiatric disorders, and cognitive impairment. We report two patients with a deletion within BP3-BP5 and two patients with smaller duplication within BP4-BP5. A 15q13.2-13.3 microdeletion (chr15:30955149-32515681) which encompasses seven protein-coding genes (*ARHGAP11B, FAN1, MTMR10, TRPM1, KLF13, OTUD7A, CHRNA7*) was detected for the 1st patient 1 y boy with hypotonia, psychomotor retardation and dysmorphic features. 2nd patient 8 y boy has a 15q13.1-13.3 deletion (chr15:29247469-32515681) which encompasses 17 protein-coding genes (*APBA2, FAM189A1, NDNL2, TJP1, GOLGA8J, GOLGA8T, CHRFAM7A, GOLGA8R, GOLGA8Q, GOLGA8H*, *ARHGAP11B, FAN1, MTMR10, TRPM1, KLF13, OTUD7A, CHRNA7*). The patient was referred to clinical geneticist due to psychomotor

retardation, ASD and dysmorphic features. 3rd patient with expressive language impairment and attention problems has 307 kb size duplication at 15q13.3 (chr15:32018731-32325676). Duplication region encompasses part of *OTUD7A* gene and part of *CHRNA7* gene. 4th patient with psychomotor delay and short stature has 194 kb duplication (chr15:32114055-323208134) involving a distal part of *OTUD7A* gene. The differential diagnosis of the 15q13.3 microdeletion/microduplication comprises an extensive spectrum of diseases. There is no consistent or recognizable phenotype. The BP4-BP5 microdeletion/microduplication events span *CHRNA7*, a candidate gene for seizures. However, none of these patients reported here have epilepsy. Both deletion and duplication encompasses *OTUD7A* gene which is critical gene for brain function.

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P08.04D

Array-CGH cohort of 1500 patients with Neurodevelopmental Disorders: Copy Number Variation in 16p13.11

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Chromosomal region 16p13.11 is structurally complex, subdivided into three single-copy sequence blocks called intervals I, II and III. Each block is flanked by low-copy repeats (LCRs) with highly homologous DNA sequences making it prone to non-allelic homologous recombination (NAHR) being a major source of *de novo* genomic rearrangements. 16p13.11 copy number variants (CNVs) have variable sizes (0,8 to 3,3Mb), encompassing one or more of the three intervals. Interval II (chr16:15.48-16.32Mb, GRCh37/hg19) CNVs, have the higher number of patients reported so far, involving a set of eight genes, including *NDE1*, referred as a strong candidate gene for neurodevelopmental disorders. Clinical features of patients with microdeletions or microduplications at chromosome 16p13.11, have been associated with a range of neurodevelopmental disorders including autism spectrum disorders (ASD), attention-deficit hyperactivity disorder (ADHD), intellectual disability (ID) and schizophrenia. In our cohort of 1500 patients, with ID, ASD and congenital anomalies, studied by Agilent 180K oligonucleotide array-CGH, we have identified 17 patients with 16p13.11 CNVs (9 deletions and 8 duplications). The majority of the patients showed high clinical variability with a wide range of phenotypic manifestations: developmental delay, autism, speech delay, learning difficulties, behavioural problems, epilepsy, microcephaly and physical dysmorphisms. In our data, an higher male:female 16p13.11 CNV ratio has not been detected. In 66% of the cases, the alteration was inherited from unaffected parents confirming that duplications and deletions at 16p13.11 represent incomplete penetration but that predispose to a range of neurodevelopmental disorders.

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P08.05A

Novel *ADAT3* variants associated with RNA modification defects and autosomal recessive neurodevelopmental disorders

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Post-transcriptional modifications of tRNAs are important for the regulation and efficiency of translation as well as for fidelity and stability of the tRNA structure. Defects in these modifications have been implicated in human diseases such as neurological and mitochondrial disorders, diabetes and cancer.

The conversion of adenosine (A) to inosine (I) of the first base in the anticodon of tRNA enables alternative pairing with U, C or A at the wobble position of mRNA codons. The A to I conversion is catalysed by a heterodimeric adenosine deaminase complex consisting of the ADAT2 and ADAT3 subunits. To date, only a single homozygous founder mutation, c.430G>A; p.Val144Met, has been detected in *ADAT3*. The main features of these patients are cognitive impairment, strabismus, hypotonia and spasticity or epilepsy.

Using exome sequencing, we identified novel compound heterozygous variants in *ADAT3* in two different non-consanguineous families, and the homozygous founder mutation in one consanguineous family. The variants c.[587C>T];[820C>T] (p.[Ala196Val];[Gln274*] were detected in three affected siblings, and c.[928_936del];[946C>G] (p.[Cys310_Met312del];[His316Asp] in a single case. All four cases share many of the features presented by the previously detected founder mutation. The mutated amino acids are highly conserved, and Sanger sequencing of cDNA from the three affected siblings shows a significantly lowered expression of the allele harbouring the nonsense variant. Further functional studies are ongoing to investigate the pathogenicity of these variants.

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P08.06B

Whole-exome sequencing of patients with Angelman-like phenotypes and no aberration detected in the *UBE3A* gene

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Introduction: Angelman syndrome (AS) is a neurodevelopmental disorder characterised by moderate to severe developmental delay, absent or near absent speech, gait ataxia, microcephaly and seizures. Deficient expression or function of the maternally inherited *UBE3A* allele results in AS. Approximately 5-10% of patients with a presumed diagnosis of AS do not have an identifiable molecular cause. A proportion of these patients may have a defect of maternal *UBE3A* expression which is not detectable by current test methods. However, there are other rare syndromes which have clinical features that overlap with AS. Whole-exome sequencing (WES) may have diagnostic utility in these patients.

Methods: Singleton WES was performed on a retrospective cohort of 37 patients with a clinical diagnosis of AS or a syndromic intellectual disability with clinical feature(s) in common with AS. None of the patients had an identified *UBE3A* aberration, and all had normal microarray test results. Variant analysis was restricted to a curated list of 812 genes associated with Angelman syndrome, intellectual disability and related neurodevelopmental disorders.

Results: Using ACMG-AMP variant-interpretation guidelines, a pathogenic or likely pathogenic variant was identified in the 14/37 patients (37.8%). Variants were detected in the *ARID1B*, *BCL11A* (2 patients), *CDKL5*, *DEAF1*, *GABRA1*, *HDAC8*, *HIVEP2*, *KAT6A*, *NEXMIF* (*KIAA2022*), *PIGN*, *POGZ*, *PURA* and *TRAPPC9* genes.

Conclusion: Results from this cohort advocate the utility of WES, following microarray and *UBE3A* testing, for patients with Angelman-like phenotypes or neurodevelopmental disorders with unclear or atypical clinical phenotypes.

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P08.07C

Whole exome sequencing identifies new genes responsible for Angelman-like syndrome

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Introduction: Approximately 10% of patients with an Angelman syndrome (AS) phenotype remain without a molecular diagnosis. Some of these AS-like syndrome patients may harbor alternative genetic defects that present overlapping clinical features with AS. Whole-exome sequencing (WES) has been successfully applied to identify the genetic bases of intellectual disability and autism.

Materials and Methods: 17 patients who met the consistent clinical features of AS and lack a molecular diagnosis were selected. WES was performed in 17 parents-patient trios in a Hiseq2000 platform (Illumina) using the SureSelectXT Human All Exon V5+UTR (Agilent). Variants were filtered according to their allele frequency in the ExAC database and their effect on the protein. Pathogenicity of missense variants was evaluated using bioinformatics tools.

Results: Candidate variants were identified in 14 of 17 patients. Ten of those variants were de novo. According to the recommendation of the ACMG/AMP, six pathogenic variants were identified in *SATB2, SYNGAP1, ASXL3, SLC6A1*, *SPTAN1* and *SMARCE1* genes whereas four likely pathogenic missense variants were identified in other neurodevelopmental genes. Clinical reevaluation is being performed in those patients with pathogenic and likely pathogenic variants.

Conclusion: Exome sequencing has proved a valuable tool to identify the genetic defect in AS-like patients. Newly identified genes should be added to the expanding list of differential diagnoses for patients presenting with AS-like features. We thank Instituto de Salud Carlos III (PI16/01411), Asociación Española de Síndrome de Angelman and Fundació Parc Taulí-Institut d'Investigació i Innovació ParcTaulí I3PT for their financial support.

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P08.08D

De novo variants disruting the HX repeat motif of ATN1 cause a non-progressive neurocognitive disorder with recognisable facial features and congenital malformations

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Polyglutamine expansions in the transcriptional co-repressor *ATN1*, at 12p13.31, have been linked to the neurodegenerative condition dentatorubral-pallidoluysian atrophy (DRPLA) via a proposed toxic gain of function. We present detailed phenotypic information on seven unrelated individuals with *de novo* missense and in-frame insertion variants within an evolutionarily conserved 16 amino acid 'poly HX repeat' motif of *ATN1*. The subjects have severe cognitive impairment, hypotonia, a recognisable facial gestalt and variable congenital anomalies but lack the progressive symptoms typical of DRPLA. We show that a variant in ATN1's HX repeat is sufficient to perturb the structural features of the repeat with several

effects. It alters ligand binding, including the binding to several proteins which are important in ribosomal function and regulation of gene expression. In addition, the mutation affects the nuclear localization of the HX repeat motif. These data suggest that the variant affects the transcriptional repression activity of ATN1, leading to an apparent gain of function effect. Our study provides valuable insights into the function of the HX repeat motifs and *ATN1's* primary roles regulating neuronal and other organ system development. This research provides an example of phenotypically distinct allelic disorders in humans, revealing the power of unbiased genomic technologies and international collaborations in providing diagnoses for individuals with complex congenital disorders.

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P08.09A

Dias-Logan syndrome: delineating a newly recognized disorder of transcriptional regulation

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Introduction: Dias-Logan syndrome is a recently described condition characterized by intellectual disability (ID) and persistence of fetal hemoglobin (HbF). It is caused by haploinsufficiency of *BCL11A* (2p16.1), encoding a transcription factor of the SWI/SNF chromatin remodeling complex.

Methods: We reviewed the medical records of our patients with changes in *BCL11A* and those in the literature, and assessed the frequency of the main manifestations.

Results: Our patients (2-11 y) presented with hypotonia (6/6), ID (6/6), persistence of HbF (3/3), brain abnormalities (4/6), strabismus (4/6), and seizures (2/6). No birth defects were observed. Two had *de novo* deletions and four had a *de novo* pathogenic variant in *BCL11A*.

By review of the literature, we found 16 additional individuals with point mutations and 25 with 2p15p16.1 microdeletions. Including our patients, the most frequent manifestations were ID (100%), varying from mild to profound, persistent HbF (100%), distinctive facial features (95%), hypotonia (87%), microcephaly (67%), abnormal brain MRI (67%), consisting of cortical dysplasia, corpus callosum hypoplasia or cerebellar hypoplasia, and growth delay (41%). Epilepsy was present in 16%: age at onset ranged 2 m - 3 y, and seizures were mostly drug-resistant. Interestingly, in most of the patients the facial gestalt resembled Alfa Thalassemia Intellectual Disability (ATRX), caused by mutations in a gene encoding another SWI/SNF-like protein.

Conclusions: Our study expands the phenotype of *BCL11A* mutations to include early onset seizures and brain abnormalities, and the overlapping manifestations between Dias-Logan syndrome and ATRX suggest convergence on a common pathway of transcription regulation of hemoglobin genes.

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P08.10B

Telomere shortening in Down syndrome and cerebral palsy

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Intellectual disability has a global prevalence of 1-3%. Down syndrome and Cerebral Palsy are two entities associated to intellectual disability with high impact in society and easily diagnosed. In recent decades, life expectancy in people with intellectual disability has increased and has been accompanied by premature aging whose genetic cause remains unknown. Telomere shortening is involved in the cellular and body aging. We have studied by quantitative real time PCR the telomere length in subjects with Down syndrome(66 males, 47 females, age 11-69y, mean 37.2) and Cerebral Palsy (34 males, 20 females, age 11-80y, mean 32.7), and compared with a control group in order to identify differences that could explain the accelerated aging of both pathological groups. We found differences in telomere length between subjects with Down syndrome and Cerebral Palsy for ages over 35y and when compared both groups to healthy subjects matched by age for all ages ($p < 0.001$) in all cases. The analysis of the genotype distribution of two polymorphisms associated with a low telomerase activity: TERT -1327C>T (rs2735940) and TERC -63G>A (rs2793607) did not show any difference between groups. Analysis of telomere length in siblings with a similar age to those of individuals with cerebral palsy as well as those of their parents corrected for age showed shorter telomeres length than control subjects with similar age. These data suggest that early telomere shortening could increase susceptibility to cerebral palsy or that both entities could share genetic predisposition factors.

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P08.11C

NGS panel for chromatinopathies, implications for diagnosis and research

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Introduction: Aberrant structure and function of chromatin, by altering various components of the epigenetic machinery, causes a number of human diseases. Intellectual disability appears to be a common phenotype feature, although these disorders affect multiple organs. There is emerging importance recognition of this spectrum of disorders, that we termed 'chromatinopathies'.

Materials and Methods: We generated a targeted NGS custom-made gene panel to sequence 66 genes that are causative of 54 chromatinopathies, including Kabuki, Au-Kline, Charge, Wiedemann Steiner, Rubinstein-Taybi, Floating Harbor, and Cornelia de Lange syndromes.

Results: To date we have NGS-sequenced 165 patients and we found pathogenic variants in 31% of the all patients. The main sub-group is Kabuki syndrome patients for which we have analysed 83 patients finding 26 (16% of the total) of them carrying *KMT2D* and *KDM6A* pathogenic point pathogenic variants. Moreover we found pathogenic variants in 9/37 Rubinstein-Taybi syndrome patients, 3/11 Cornelia de Lange,syndrome 2/10 in Floating-Harbor syndrome, 2/3 Sotos syndrome, and 1/1in Wiedemann-Steiner syndrome. Then we extended our analysis searching for additional causative genes alteration in those patients with overlapping phenotypes, finding unexpected results supporting the need of using NGS for this group of diseases with molecular and clinical overlapping.

Conclusions: The study of chromatinopathies may offer a unique opportunity to learn about the role of epigenetics in health and disease. Since the pathogenic sequences are unknown for most of the cases, we highlight the importance to analyse them with NGS.

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P08.12D

Ending the diagnostic odyssey by clinical whole exome / genome sequencing (CWEW/CWGS)

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Introduction: There are more than 7,000 rare diseases affecting 300 million populations worldwide. Unfortunately, the diagnosis of rare diseases is challenging and very often, there is a delay between disease onset and the time of the correct diagnosis. This is also known as "diagnostic odyssey". Over the past years, we had closed a number of "diagnostic odysseys" in this locality and we realized there is a strong need to provide rare diseases diagnostic service. For this reason, we developed the first Undiagnosed Diseases Program (UDP) in Hong Kong which was supported by the S. K. Yee Medical Foundation.

Materials and Methods: Clinical Whole Exome and Genome Sequencing (CWES and CWGS) were performed with bioinformatics analysis done using in-house algorithm. The overall interpretation was based on the clinical, laboratory and imaging findings, and pathomechanism.

Results: Over 100 cases had been referred to us and the disease entities were heterogeneous. Some cases were potentially actionable, these include Allan-Herndon-Dudley syndrome, benign recurrent intrahepatic cholestasis (BRIC), coenzyme Q6 deficiency-related nephrotic syndrome, coenzyme Q10 deficiency, osteogenesis imperfecta type VII, steroid-resistant nephrotic syndrome, X-linked adrenoleukodystrophy, etc. A new treatment for *GNAO1*-related epilepsy was found by this group. Novel disease-causing genes were discovered, for example, *AK9* in congenital myasthenic syndrome (CMS) and *EBF3* in Moebius syndrome.

Conclusions: Patients with undiagnosed disease >3 months should undergo CWES/CWGS. The clinical interpretation of CWES/CWGS is not straightforward, and requires in-depth knowledge in both advanced laboratory and clinical medicine. Therefore, it should be handled by specialists with experience in clinical genomics.

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P08.13A

The search of biological processes affected by *CNTN6* microdeletion in neurons, derived from induced pluripotent stem cells of a patient with intellectual disability and 3p26.3 microdeletion

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Introduction: Microdeletions and microduplications affecting *CNTN6* have been described in patients with neurodevelopmental disorders. We aimed to find out the basic biological processes involved in the realization of pathogenic microdeletion effects in the central nervous system (CNS) via analysis of differentially expressed genes (DEG) in neurons, derived from induced pluripotent stem (iPS) cells of a patient with intellectual disability and *CNTN6* microdeletion.

Materials and Methods: Two iPS cell clones with *CNTN6* microdeletion and three wild-type (WT) cell clones with different genotypes were differentiated into cortical neurons. The neuronal RNA was examined using SurePrint G3 Human Gene Expression 8×60K Microarray Kit (Agilent Technologies, USA). Empirical Bayes statistical test was applied for bioinformatic analysis (p-value < 0.05; expression levels differ more than 2 times). The enrichment analysis for identification of functional groups of genes was performed.

Results: The comparison of gene expression in WT and *CNTN6* microdeletion neurons revealed 756 DEG (142 downregulated and 614 upregulated genes). The enrichment analysis of the underexpressed genes revealed their involvement in responses to glucocorticoid and corticosteroid, astrocyte development, basic amino acid and transmembrane transport. Overexpressed genes were involved in regulation of synaptic signalling, trans-synaptic signalling, anterograde trans-synaptic signalling, chemical synaptic transmission, cell-cell signalling, nervous system and neuron development, regulation of postsynaptic membrane potential.

Conclusions: The transcriptome analysis of iPSC-derived neurons with the *CNTN6* microdeletion revealed the alteration of the expression of other genes significant for CNS functioning. This study was supported by Russian Science Foundation, grant 14-15-00772.

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P08.14B

Expanding the phenotype of *CTNNB1*-mutated individuals and description of the fetal phenotype

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Introduction: *CTNNB1* constitutional mutations were first described in intellectual disability by De Ligt et al. in 2012. Thirty-one individuals have been reported so far and a further 16 non-published mutated individuals are reported by the Deciphering Developmental Disorders study. The *CTNNB1* gene encodes for the highly-conserved beta-catenine which has an major role in the Wnt-signalling pathway.

Results: Using French National networks, we collected clinical data of 13 individuals (including one previously published patient) from 11 centers and 2 fetuses. Individuals were diagnosed using array-CGH, gene panels, solo or trio exome sequencing. We decribe new signs in *CTNNB1*-mutated individuals and the fetal phenotype. Thirteen have SNVs and two have deletions. New signs include condensing bone anomalies, severe acne and reproductive organ malformation. One fetus was diagnosed after termination of pregnancy for agenesis of corpus callosum and gene panel analysis. The other fetus had microcephaly (-3DS), array-CGH was performed and revealed a deletion encompassing *CTNNB1*.

Conclusion: We expand the phenotype of *CTNNB1*-mutated individuals and describe the fetal phenotype and new mutations. More patients are needed to confirm the frequency of rarer signs. X-rays in both males and females and abdominal ultrasound in females should be carried out to respectively assess bone anomalies and reproductive organ malformations.

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P08.15C

DDX3X mutations in 11 french patients with intellectual disability : new phenotypic features

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Intellectual disability affects approximately 2.5% of humans. The prevalence of DDX3X (X-linked, MIM *300160) mutations in intellectual disability (ID) is not yet known, but have been reported in 45 patients with ID. Other symptoms included hypotonia, movement disorders, microcephaly, behavior problems and epilepsy. Several additional features were noted, including hyperlaxity, skin abnormalities, cleft lip, cleft palate, hearing loss, visual

impairment, and precocious puberty. So far, all amino acid substitutions in females were de novo and localized in one of the two protein subdomains : the helicase ATP-binding domain or the helicase C-terminal. We report 11 French females patients with DDX3X mutations, carrying 8 new mutations, including one substitution outside those two domains : c.113A>G. We describe the first case of mother-daughter transmission of DDX3X mutation : c.543+2_543+3del. Those two patients are schizophrenics. All patients present ID, with speech and walk delay. Six present feeding difficulties. Two patients are obese, two others have thyroid issues. One has ASD, another one had neuroblastoma in young childhood. None of them present hearing loss.This report leads to 56 patients with DDX3X mutations report in the litterature and expand the phenotypic spectrum of DDX3X.

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P08.16D

Different mutations in *DEAF1* lead to clinically distinct dominant and recessive forms of intellectual disability

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Mutations in *DEAF1*, encoding a crucial transcription factor in central nervous system development during early embryogenesis, were reported to lead to autosomal dominant mental retardation 24 (MRD24; MIM 615828) and autosomal recessive dyskinesia, seizures, and intellectual developmental disorder (DYSEIDD; MIM 617171). We aimed at understanding genotype-phenotype correlations of 15 novel patients with likely pathogenic *DEAF1* variants identified by exome sequencing. In 13 cases, *de novo DEAF1* variants resulted in single amino acid changes in the SAND domain. Two unrelated patients had inherited compound heterozygous *DEAF1* variants, which were located outside the SAND domain and both consisted of a combination of a loss-of-function mutation with a milder mutation on the other allele. Analysis of transcriptional repression activity at the *DEAF1* promoter showed that the tested *de novo* variants impaired DEAF1 function, while this effect could not be observed for recessive variants. *De novo DEAF1* variants caused moderate/severe intellectual disability with limited or absent speech and behavioral problems (mood swings, autism, self-aggression and sleep disturbance). Recessive *DEAF1* variants resulted additionally in more severe psychomotor delay, movement disorder and MRI abnormalities. Epilepsy, occurring in recessive patients and most of dominant patients, was frequently difficult to treat. Some patients used non-verbal

communication methods, suggesting better receptive than expressive language. We provide further insight in the genotype and phenotype spectrum of *DEAF1*-related dominant and recessive intellectual disability. Detailed phenotype information, segregation and functional analysis are fundamental to determine the pathogenicity of novel variants and to improve the care of these patients.

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P08.17A

De novo variants in _SNAP25_ cause a spectrum of developmental and epileptic encephalopathy

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Introduction: Synaptosomal-associated Protein-25 (SNAP25), predominantly expressed in the brain, is part of the SNARE complex (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) required for proper presynaptic vesicle docking and fusion. Heterozygous *de novo* variants in *SNAP25* have previously been separately reported in three individuals with intellectual disability (ID), epileptic encephalopathy, ataxia and congenital myasthenia.

Results: We have collected detailed phenotypic data on at least four additional cases with *de novo* variants in *SNAP25*. Combined with the three publishes cases, all seven individuals presented with ID with three of them classified as severe, three as moderate and one as mild. Five individuals developed seizures with a spectrum of epileptic spasms, focal and generalized seizures. Four remained refractory to therapy. Three individuals did not attain walking skills by age eleven years or later. Movement disorders of dystonia or choreoathetosis were seen in two individuals. Brain imaging revealed two individuals showing generalized volume loss. In addition, one case presented with signs of a leukoencephalopathy. Further symptoms include microcephaly, ataxia, cortical visual impairment, congenital myasthenia and congenital hip dysplasia and contractures. All causative variants constitute *de novo missense* variants located in the t-SNARE coiled-coil homology domain 1 & 2, both showing a significantly reduced number of *missense* variation in controls, indicating a selective constraint.

Conclusion: *De novo* variants in *SNAP25* cause a spectrum of developmental and epileptic encephalopathy. Further patients and studies are needed to improve our understanding of the phenotypic spectrum and elucidate the effects of the variants on protein function.

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P08.18B

The role of recessive inheritance in early-onset epileptic encephalopathies: a combined whole-exome sequencing and high-resolution copy number study

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Intoduction: Early-onset epileptic (EE) and combined developmental and epileptic encephalopathies (DEE) represent a group of epilepsies characterized by generally poor outcome. A few whole exome or genome sequencing studies have emphasized the causative role of *de novo* mutations in a growing number of EE/DEE disease genes, yet leaving the majority of patients without etiological diagnosis. The aim of this study was to further elucidate the genetic etiology of EE/DEE.

Materials and Methods: We performed high-resolution chromosomal microarray analysis and whole-exome sequencing in 63 independent patients with EE/DEE. Assessment of pathogenicity included molecular modelling of missense variants and untargeted plasma-metabolomics in selected patients.

Results: We yielded a diagnosis in ~42% of cases with causative copy number variants in 6 patients (~10%), (likely) pathogenic sequence variants in 14 known and two newly confirmed disease genes in 20 patients (~32%), including compound heterozygosity for causative sequence and copy number variants in one patient. 38% of diagnosed cases were caused by recessive genes, albeit with one allele occurring *de novo* in two instances. Notably, the recessive gene *SPATA5* was found causative in 3% of our cohort but was difficult to detect and therefore may have been underdiagnosed in previous studies. We further support candidacy of four previously described genes, three of which also followed a recessive inheritance pattern.

Conclusion: Our results confirm the importance of *de novo* causative gene variants in EE/DEE, but additionally illustrate the major role of mostly compound heterozygous or hemizygous recessive inheritance and consequently high recurrence risk.

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P08.19C

De novo truncating variants in the intronless *IRF2BPL* gene are responsible for developmental epileptic encephalopathy

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Developmental and epileptic encephalopathies (DEEs) are severe clinical conditions characterized by stagnation or

decline of cognitive and behavioural abilities preceed, accompanied or followed by seizures. Because DEEs are clinically and genetically heterogeneous, next-generation sequencing - especially Whole Exome Sequencing (WES) - is becoming a first-tier strategy to identify the molecular etiologies of these disorders. By combining WES analysis and international data sharing we identified 10 unrelated individuals with DEE and *de novo* heterozygous truncating variants in the interferon regulatory factor 2-binding protein-like gene (*IRF2BPL*). The 10 individuals allowed delineation of a consistent neurodevelopmental disorder characterized by disturbed/normal initial psychomotor development followed by severe global neurological regression, usually starting in childhood, and epilepsy with non-specific EEG abnormalities and variable CNS anomalies including cerebral or cerebellar atrophy. *IRF2BPL*, also known as enhanced at puberty protein 1 (*EAP1*), encodes a transcriptional regulator containing a C-terminal RING-finger domain common to E3 ubiquitin ligases. This domain is required for its repressive and transactivating transcriptional properties. The variants identified are expected to encode for protein lacking the C-terminal RING-finger domain. Taken together these data support the causative role of truncating *IRF2BPL* variants in paediatric neurodegeneration and expands the spectrum of transcriptional regulators identified as molecular factors implicated in genetic developmental and epileptic encephalopathies.

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P08.20D

FBXL3, novel candidate for autosomal recessive intellectual disability

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The study of consanguineous families has proven valuable in the identification of novel causative genes for monogenic disorders. In a large study of consanguineous families focusing in intellectual disability, we combined exome sequencing and homozygosity mapping and identified two consanguineous families with homozygous loss-of-function variants in *FBXL3*. In the first family, from Lebanon, *FBXL3* was the only gene among five candidates with a loss of function variant (NM_012158.2:c.445C>T:p.(Arg149*)); and in the second family, from Pakistan, the *FBXL3* variant (NM_012158.2:c.884del:p.(Leu295Tyrfs*25)) was the only one segregating in five affected individuals in two family loops. In both families, the patients presented with intellectual disability, short stature and mild facial dysmorphism, a relatively large nose with bulbous tip. Fbxl3 variants have been engineered in mice and are associated with disturbances of the circadian rhythm and behavioral problems. Consanguinity provides the opportunity to identify novel candidate genes for various phenotypes and enhance the diagnostic yield of mendelian disorders.

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P08.21A

Diagnostic yield of exome trio analysis to identify the genetic etiology in 404 undiagnosed cases

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Background: Exome trio analysis is an effective strategy to identify potentially causal variants, along with their inheritance pattern, on rare genetic disorders. This approach has entered the medical practice as an effective diagnostic test transforming the molecular diagnosis and clinical management of undiagnosed genetic diseases.

Material And Methods: We performed exome sequencing using Ion AmpliSeq™ Exome RDY technology (Life Technologies) and SureSelect^{XT} Human All Exon V6 technology (Agilent Technologies). Sequencing reads were analyzed using Torrent Suite software and an in-house pipeline, respectively. Trio annotated variants using ION

Reporter were prioritized with an in-house analytical pipeline.

Results: We present the analysis of 404 trios referred to a single institution. Patients were mainly children with syndromic intellectual disability (46%). The genetic etiology was potentially elucidated in 129 probands harboring 82 causal variants and 47 likely causative variants, achieving a 32% genetic diagnostic rate. Among these patients, 80 harbored *de novo* variants, 12 hemizygous maternally inherited variants, 10 in compound heterozygous variants, 21 newly homozygous variants and 6 variants inherited from parents. Patients with syndromic intellectual disability (42%, 78/187) and specific neurological disorders (40%, 19/48) showed higher molecular diagnostics rates than patients with non-neurologic disorders (26%, 8/31) and non-syndromic intellectual disability (17%, 24/138).

Conclusions: In our cohort exome trio analysis provide a diagnostic yield of 32% in patients whom traditional molecular diagnostics strategies were uninformative. The implementation of exome trio analysis *as a first-tier diagnostic approach* will provide a higher diagnostic yield and a cost-efficient option particularly in rare syndromic intellectual disabled patients.

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P08.22B
Biallelic loss-of-function mutations of *EZH1* may cause novel developmental disorder

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Introduction: EZH1 is a component of a Polycomb repressive complex-2 (PRC2) that mediates methylation of H3K27. It has important roles in the maintenance of embryonic stem cell pluripotency and plasticity. Mutations of PRC2 subunits often cause over growth and neurological diseases. Weaver syndrome is characterized by overgrowth, intellectual disability (ID), and characteristic facial features. Mutations in EZH2 have been identified as a cause of Weaver syndrome. We report sisters with biallelic loss-of-function mutations of ***EZH1***. Clinical report: (Patient 1) The 7-year-old female was the first child of healthy and non-consanguineous Japanese parents. She was hypotonic and her development was delayed. She showed moderate ID. Physical examination revealed no dysmorphic features. At 6 years of age, she showed accelerated growth. She was diagnosed with precocious puberty. (Patient 2) The 5-year-old female was the younger sister of patient 1. Her development was delayed. She stated to walk alone after 3 years old. She spoke no meaningful words. She showed severe ID. Physical examination revealed no dysmorphic features.

Methods: With the approval of our institutional ethics committee, the samples were analyzed using WES.

Results: Biallelic loss-of-function mutations of ***EZH1*** were found in the sisters. Their parents and unaffected sister was heterozygous for the mutation.

Conclusions: Probability of being LoF intolerant for ***EZH1*** is very low. Heterozygous deletion of ***EZH1*** was non-candidate for developmental disorder. Haploinsufficiency of EZH1 may not cause abnormal methylation of H3K27. Our results show that biallelic loss-of-function mutations of ***EZH1*** may cause developmental disorder with precocious puberty.

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P08.24D
Clinical and molecular characterizationof three cases of Floating-Harbor syndrome

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Introduction: Floating-Harbor syndrome (FHS) (MIM# 136140) is a very rare condition characterized by short stature with delayed bone age, expressive language delay and typical facial dysmorphism. FHS is due to heterozygous pathogenic variants in the *SRCAP* gene. We present three cases of FHS. Our aim is to highlight the clinical and molecular features of this syndrome.

Cases description: We present three patients, aged 5, 10 and 11 years old, all with proportionate short stature with delayed bone age, relative macrocephaly, and mild to

moderate intellectual disability/ developmental delay with language impairment. Other common features included recurrent respiratory infections, hypotonia in the neonatal period, brachydactyly, 5th finger clinodactyly, high-pitched voice and dental problems. One of the patients presented also deafness, constipation, Arnold-Chiari malformation, and absence seizures. Two cases were medicated with growth hormone (GH). In all cases, the diagnosis was suggested by the facial gestalt and confirmed by the identification of the c.7303C>T, p.(Arg2435*) or c.7330C>T, p.(Arg2444*) pathogenic variants in the *SRCAP* gene.

Conclusions: FHS should be considered in the differential diagnosis of short stature and language developmental delay, and can be recognized by the typical facial gestalt. We report the second case of FHS with Arnold-Chiari malformation and a case with toenails hypoplasia. The use of GH is controversial, and should be evaluated in these cases. Long term follow-up is needed to understand the evolution of the phenotype of patients with this very rare entity.

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P08.25A

Combination therapy in fragileX syndrome; possibilities and pitfalls illustrated by targeting themGluR5 and GABA pathway simultaneously

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Fragile X syndrome (FXS) is the most common monogenetic cause of intellectual disability and autism. The disorder is characterized by altered synaptic plasticity in the brain. Synaptic plasticity is tightly regulated by a complex balance of different synaptic pathways. In FXS, various synaptic pathways are disrupted, including the excitatory metabotropic glutamate receptor 5 (mGluR5) and the inhibitory γ-aminobutyric acid (GABA) pathways. Targeting each of these pathways individually, has demonstrated beneficial effects in animal models, but not in patients with FXS. This lack of translation might be due to oversimplification of the disease mechanisms when targeting only one affected pathway, in spite of the complexity of the many pathways implicated in FXS. In this report we outline the hypothesis that targeting more than one pathway simultaneously, a combination therapy, might improve treatment effects in FXS. In addition, we present a glance of the first results of chronic combination therapy on social behavior in *Fmr1* KO mice. In contrast to what we expected, targeting both the mGluR5 and the GABAergic pathways simultaneously did not result in a synergistic effect, but in a slight worsening of the social behavior phenotype. This does implicate that both pathways are interconnected and important for social behavior. Our results underline the tremendous fine-tuning that is needed to reach the excitatory-inhibitory balance in the synapse in relation to social behavior. We believe that alternative strategies focused on combination therapy should be further explored, including targeting pathways in different cellular compartments or cell-types.

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P08.26B

diagnostic approach with genetic tests of global developmental delay and/or intellectual disability: single tertiary centre experience

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Diagnostic approach with genetic tests of global developmental delay and/or intellectual disability

Background: Global developmental delay (GDD) and intellectual disability (ID) are common cause for referral to pediatric department. Recent advance in genetic tests have resulted that multiple diagnostic approach including brain imaging, metabolic tests, or genetic tests is possible for children with GDD or ID.

Methods: We retrospectively reviewed the medical records of children with GDD/ID attending the pediatric neurology department of Daejeon St. Mary's Hospital during the period from January 2016 through March 2017.

Results: A total of 75 children were investigated for GDD/ID in the pediatric neurology departments. Ten patients (13%) were diagnosed with specific disease such as Rett syndrome, Prader Willi syndrome. Chromosomal microarray was performed as 1st tier test and 25 patients (33%) diagnosed. Brain structural abnormalities were showed 8 patients (11%) and two patients diagnosed Fragile X syndrome. Thirty patients who did not revealed etiology of GDD/ID received gene panel by next generation sequencing. Eight patients were found out underlying genetic etiology: *CHD8*, *ZDHCC9*, *CACNA1H*, *SMARCB1*, *FOXP1*, and *KCNK18*. However, twenty two patients (29%) were remained uncertain cause of GDD/ID.

Conclusion: This study provides information on pediatricians to diagnostic approach of children with GDD/ID. Early detection of detection of GDD/ID can help treatment planning and assignment of the recurrence risk for siblings, and emotional relief for the family. Practicality, precision,

yield, and genome-based diagnosis in medical care must be defined in future researches and will need prospective study designs.

J. Han: None.

P08.27C

Helsmoortel-Van der Aa Syndrome as emerging clinical diagnosis in intellectually disabled children with autistic traits and ocular involvement

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Introduction: A recent syndromic condition with craniofacial dysmorphisms, comprising congenital ocular defect and neurodevelopmental delay named Helsmoortel-Van der Aa Syndrome (HVDAS)(OMIM#615873), has been described and molecularly defined, identifying pathogenic mutations in the *ADNP* gene (OMIM#611386) as biological cause.

Materials and Methods: We report on two children, displaying intellectual disability (ID) and peculiar congenital eyes anomalies, referred to a clinical genetics evaluation to better define their condition.

Results: Both patients resulted to carry a *de novo* nonsense mutation in the *ADNP* gene, identified by Next Generation Sequencing analysis (NGS).

Conclusions: The review of present and literature reports, suggests that the diagnosis of HVDAS should be suspected in patients with ID accompanied by behavioral features in the Autism Spectrum Disorder and distinctive craniofacial phenotype. Among dysmorphisms due to malformation of the periorbital region, ptosis appears to be particularly recurrent in HVDAS. Furthermore, the present patients could support the inclusion of the HVDAS associated with specific mutations clustering within a small *ADNP* genomic region among clinical conditions reminiscent of the blepharophimosis/mental retardation syndromes (BMRS).

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P08.28D

Identification of novel variants in *HNRNPU* associated with global developmental delay, epilepsy and multiple organ system defects

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Abstract

Background: *HNRNPU* (OMIM *6022869) encodes a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family which mediates different aspects of RNA transport and metabolism by forming ribonucleoprotein complexes in the nucleus. Heterozygous *HNRNPU* variants have been associated with global developmental delay, seizures and autistic features; additional symptoms such as cardiac and renal abnormalities as well as dysmorphic features have been reported.

Case Presentation: Here we report the identification of previously unreported *de novo* variants in two index patients presenting with intellectual disability, speech impairment and epilepsy. Additional clinical features included cardiac defects, recurrent infections, hypermobility of the joints, muscular hypotonia and brain atrophy.

Methods: We performed exome sequencing on the index patients. Variant confirmation and carrier testing was done by Sanger sequencing.

Results: Exome sequencing and downstream variant prioritization led us to identify predictively truncating *de novo* Variants in *HNRNPU* including a heterozygous stop_variant (c.1801C>T, p.Arg601*) and a frameshift variant (c.974del, p.Ala325Alafs*14).

Conclusion: Here we report on the phenotypic features associated with novel truncating *de novo* mutations in *HNRNPU*. Global developmental delay and epilepsy present as common features observed in all *HNRNPU* cases reported to date. Apart from the central nervous system additional organ systems appear to be variable affected. Our findings support the role and implication of *HNRNPU* in the development and functions of different body organs in addition to the central nervous system.

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P08.29A

A novel *HS6ST2* variant reduces the enzyme activity in two brothers with severe myopia and syndromic intellectual disability

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Introduction: An Italian family composed of healthy non-consanguineous parents, two affected male children and one healthy son came to our attention. The affected sibs presented with febrile seizures, EEG irregular diffuse spike-wave anomalies, severe myopia, mild facial dysmorphisms and developmental delay. Their phenotypic features resembled those associated with Brooks Wisniewski Brown (BWB) syndrome even though probands were wild type for *HUWE1*, described in literature as causative of BWB.

Material and Methods: WES of all the family members was set up. To evaluate the effect of the identified variant, site-directed mutagenesis was performed on a commercial expression vector containing HS6ST2 cDNA. Transient expression of both wild type and mutant transcripts was carried out in HEK293 cells and HS6ST2 enzymatic activity was assayed.

Results and Conclusion: WES highlighted the novel maternally-inherited mutation c.916 G>C (G306R) at *HS6ST2* gene, present in hemizygous state in the two probands and absent in their healthy brother. *HS6ST2* maps at Xq26.2, a locus associated with X-linked mental retardation and recessive myopia, and encodes a member of the heparan sulfate (HS) sulfotransferase (ST) family expressed in brain and eye during development. c.916 G>C variant affects *HS6ST2* substrate binding site and its effect was considered "deleterious" by many *in-silico* tools. *In-vitro* enzymatic assay revealed that HS6ST2 mutant isoform maintained 36% of transferase activity, supporting our hypothesis of its involvement in the pathological phenotype establishment.

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P08.30B

HUWE1 gene variants as the cause of snydromic or nonsyndromic intellectual disability: spectrum of the *HUWE1* associated phenotypes

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Pathogenic variants in *HUWE1* have been described as the cause of a broad spectrum of phenotypes ranging from isolated craniosynostosis to non-syndromic or syndromic

X-linked intellectual disability (ID). Some of the syndromic cases were clinically diagnosed as the Brooks, Juberg-Marsidi or Kabuki-like syndromes. To our knowledge, 16 families with *HUWE1* mutations have been reported, and all variants were missense. No genotype-phenotype correlation has been identified yet. We present a novel family with a missense *HUWE1* variant NM_031407.6:c.12195G>C p.(Trp4065Cys) in two male cousins. Both boys suffered from ID, epilepsy and autism, and showed similar facial phenotypes with epicanthus, strabism, short palpebral fissures, low-set dysplastic ears, prominent nose and broad columella. One of the boys had a short stature and failure to thrive. Their facial phenotype was also very similar to patients with a neighboring variant (p.(R4063Q), Friez et al., 2016)), but not to the other previously published cases whose variants were more distant. To our knowledge, autism has not been associated with *HUWE1* yet, in contrast to craniosynostosis, which has been reported repeatedly but was not present in our patients. Interestingly, craniosynostosis has been described only in patients carrying different amino acid substitutions in position 110. Our report contributes to the delineation of the *HUWE1* phenotypic spectrum which is apparently broad and seems not to be strongly influenced by the localization of the variant, with a possible exception of craniosynostosis. The phenotypic heterogeneity must be considered in interpretation of exome sequencing data. Supported by MH CR AZV 15-33041A, 17-29423A and 00064203.

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P08.31C

X-linked intellectual disability: report on 6 families with *HUWE1* missense mutations and literature review

S. M. Ribeiro[1], J. Sá[1], A. Beleza[1], C. Reis[1], F. Ramos[1], P. Louro[1], S. Maia[1], H. Williams[2], M. Harakalova[3], G. Van Haaften[3], M. Simões[4], C. Barroso[4,5], H. Froufe[4], C. Egas[4,5], J. M. Saraiva[1,6], S. B. Sousa[1,6], M. Venâncio[1,6]

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Introduction: Most of the over 200 phenotypes associated with X-linked intellectual disability (XLID; 10% of cases of ID in boys) are not specific and next-generation sequencing techniques are crucial for their accurate diagnosis. *HUWE1* missense variants have been described as causative in a limited number of XLID families with wide phenotypic variability.

Methods: Clinical and molecular characterization of all cases with *HUWE1* point mutations identified in our department through retrospective analysis of medical records and literature comparison.

Results: We report 9 affected males from 6 unrelated families, each with a distinct *HUWE1* missense variant identified. One variant had already been reported and was considered likely pathogenic. Five were novel: 1 was considered as likely pathogenic, 3 as of uncertain significance and 1 as benign due to its presence on the unaffected brother (case excluded). One was *de novo* and 5 were inherited. The X-inactivation patterns were normal in 3/5 heterozygous mothers.

Conclusions: Our clinical findings are in accordance with the literature. All patients had significant global development delay / ID with limited speech and variable dysmorphisms. One had pre- and postnatal severe short stature and postnatal microcephaly. Features not previously reported include a multicentric ganglioneurocytoma and a linear hypopigmented nevus. Our results reinforce the likely pathogenic role of *HUWE1*-missense variants in XLID and the difficulty in interpreting novel variants in this gene.

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P08.33A

Impact and rates of exonic *de novo* mutations in patients with intellectual disability

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Due to extensive locus heterogeneity the causes of intellectual disability are numerous and unknow in many cases. For such spectrum of disorders the variation of *de novo* mutations (DNMs) are less frequent and potentially more deleterious, could offer insights into risk-determining genes or integrated deleterious effect of DNMs. Accordingly, the main interest of this study was to evaluate the observed rate and consequence of *de novo* point and indels mutations in the exomes of ID subjects and their sibs. The data set consisted of the Lithuanian patients with ID and relevant family members samples. Sequencing data of 9 parent-offspring trios exomes generated by sequencer SOLiD 5500™ and 33 more parent-offspring and 16 parent-sibs trios exomes were generated by Illumina™. Considering platform of sequencing primary analysis performed by Lifescope™ and GATK™ respectively. DNMs called by VarScan. Called potentially DNMs were filtered, manually reviewed by the IGV and validated by Sanger sequencing. Functional annotation of all identified DNMs were performed using ANNOVAR. The findings indicate that the number of DNMs varies between 1 and 12. Preliminary results of single nucleotide DNM rate before validation step is 3.1×10^{-6}, 95% CI [$2.2\text{x}10^{-6}$; $4.2\text{x}10^{-6}$], and *de novo* rate for indels - 1.1×10^{-6}, 95% CI [$5.4\text{x}10^{-8}$; $5.7\text{x}10^{-6}$]. Functional analysis revealed rs398123009 (CM1211547), rs121909121 (CM072075), rs28934906 (CM992178) DNMs that determine Schuurs-Hoeijmakers (OMIM: 615009), Pitt-Hopkin (OMIM: 602272) and Rett syndrome (OMIM: 312750). Functional analysis of remaining DNMs, their clusters and hot spots are ongoing. This study supported by the Lithuanian-Swiss cooperation program under UNIGENE project agreement no. CH-3-ŠMM-01/04.

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P08.35C

FAAH2 gene disruption in a female with intellectual disability and epilepsy bearing an X-autosome translocation

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Introduction: *FAAH2* encodes a fatty acid amide hydrolase that plays a role in the complex neural endocannabinoid signaling system. *FAAH2* gene maps to chromosome Xp11 and has been suggested as a possible candidate gene for X-linked intellectual disability (XLID). We present a female patient with intellectual disability (ID) and epilepsy, bearing a *de novo* translocation spanning *FAAH2* gene.

Patient and Methods: 34-years old female proband with mild-moderate ID and absence and generalized seizures, since the age of 7 years. Conventional and molecular karyotypes were performed. FISH experiments were completed using BAC clones for breakpoint's approach. Rearrangement breakpoints were characterized by whole genome sequencing and validated by Sanger sequencing. X-inactivation studies were also performed.

Results: Cytogenetic analysis showed an apparent balanced translocation [46,XX,t(X;10)(p11.2;q25)dn] and absence of cryptic genomic imbalances. Translocation breakpoints were narrowed by FISH between RP11-466I19 and RP11-613D18 probes at 10q25, and RP11-141B06 and RP11-497J09 at Xp11.2. Sequencing analysis revealed two disrupted genes at translocation breakpoints: *FAAH2* (Xp11.2) and *NHLRC2* (10q25). The normal X-chromosome was > 90% inactive.

Conclusions: Our patient displayed a skewed pattern of X-inactivation of the normal X-chromosome, silencing the normal *FAAH2* gene, while the disrupted allele is predicted to render a non-functional protein. No mutations in the *NHLRC2* gene or associated phenotypes have been reported in humans and animal models suggest embryonic lethality in recessive inheritance. We, therefore, conclude that the *FAAH2* gene is likely to play an important role in neurodevelopment, causing XLID and epilepsy.

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P08.36D

Intellectual disability, hypotonia and episodes of unexplained hyperthermia: think *COG7*

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Congenital glycosylation deficits (CDG syndromes) are a group of pathologies caused by a defect of synthesis of glycoproteins. *COG7* is a gene encoding one of the subunits of the Golgi oligomeric complex (COG), involved in intracellular trafficking and modification of glycoproteins. The pathogenic variants of *COG7* cause CDG type -IIe, resulting in abnormalities of the N- and 0-glycosylation pathway. So far, only eight cases have been reported in the literature. We describe two sisters from a Moroccan consanguineous couple and review the literature. These two sisters present a phenotype associating moderate to severe intellectual impairment with abnormalities in cerebral MRI, unexplained episodes of fever with macrophagic activation syndrome-like, hepatomegaly and growth retardation. They are also insensitive to pain in the extremities, which has never been described before. Through the association of homozygosity mapping and exome sequencing, we have highlighted a homozygous intronic variant c. 170-7A>G in the *COG7* gene, previously reported in a consanguineous family evoking a founder effect of this variant. Biochemically, the electrophoresis of transferrin on a Guthrie blood spot was normal and only the 2D isoform analysis of transferrin and apoC3 were able to confirm the diagnosis of CDG type- IIe. This observation of a consanguineous family allows the identification of two new cases of CDG type -IIe with the variant c. 170-7A>G in the *COG7* gene. We extend the phenotype of CDG syndromes caused by *COG7* and show the importance of transferrin and ApoC3 analysis in 2D electrophoresis for the diagnostic orientation of CDG syndromes.

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P08.37A

Medical exome sequencing vs whole exome sequencing in the diagnosis of intellectual disability

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More than 1000 genes are known in intellectual disability (ID) and new genes are still discovered monthly. Because of this genetic heterogeneity, Next Generation Sequencing (NGS) has allowed great progress.

We included 676 probands with ID and their parents after clinical evaluation and negative testing, i.e array CGH, Fragile X and, for some cases, targeted sequencing or small NGS panels. We performed medical exome sequencing (MES) with the Illumina TruSight One (including 4813 genes known in pathology) for 262 patients. And then we performed whole exome sequencing (WES) with the Roche Medexome for 464 patients, including 50 patients with negative MES.

We made 55/262 (21%) diagnoses with MES and 202/464 (44%) with WES. 38% of the diagnostic variants identified with WES were in genes absent from MES because they were too recently discovered. Besides, we found 17/50 (34%) diagnoses with WES in patients with no diagnosis with MES. Moreover, we identified 25 additional likely pathogenic variants with WES in yet unpublished genes (ongoing international collaborations). All diagnoses were validated through strong collaboration between clinical and biological geneticists.

Thus nearly 40% of our WES diagnoses would not have been found with MES, although MES theoretically included all known ID genes when it was designed. This is explained by the discovery of many new genes in ID over the past 3 years, which makes a panel of genes rapidly obsolete whereas WES remains always up to date and sees its diagnostic rate increase with scientific advances.

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P08.39C

***De novo FBXO11* mutations are associated with intellectual disability, microcephaly and behavioural anomalies**

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Intellectual disability (ID) has an estimated prevalence of 1.5-2% and in most affected persons its genetic basis remains unclear. Whole exome sequencing (WES) has proven to be a valuable tool to identify causative gene defects and has shown that a large proportion of sporadic ID cases results from *de novo* mutations.

Here, we present two unrelated patients with common clinical features and deleterious *de novo* variants in *FBXO11* detected by WES. Patient 1 has mild ID, mild microcephaly, hyperkinetic disorder, corrected cleft lip and alveolus, mild brain atrophy and mild dysmorphism. Trio WES detected a heterozygous *de novo* 1bp insertion in the splice donor site of exon 3 which is predicted unequivocally to result in aberrant splicing. Patient 2 showed ID, growth retardation, mild microcephaly, hyperkinetic and restless behaviour, as well as mild dysmorphism. WES detected a heterozygous *de novo* nonsense mutation.

FBXO11 (Homo sapiens F-box protein 11) encodes a member of the F-box protein family which form part of the SCF ubiquitin ligases. Two ID patients with *de novo* variants in *FBXO11* are mentioned without additional clinical data by Lelieveld et al. (Nat Neurosci. 2016;19:1194-6). Only one patient with clinical information and a *de novo FBXO11* mutation has been reported by Martínez et al. (JMG 2017;54:87-92). Interestingly, this patient carries the identical mutation as our patient 2 and also displays ID, growth retardation, microcephaly, behavioural anomalies, and dysmorphisms. Thus, we propose deleterious *de novo* mutations in *FBXO11* as a novel cause of ID and possibly microcephaly and behavioural anomalies.

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P08.41A

Whole exome sequencing reveals a novel missense mutation in the *ZNF674* gene, underpinning its association with X-linked mental retardation

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Background: Numerous genes have been implicated in non-syndromic mental retardation. The association of ZNF674 gene mutations in isolated X-linked intellectual disability is controversial. While some studies have shown a clear relationship between mutations and deletions in ZNF674 gene with mental retardation, other authors have contradicted these findings. Our report presents a novel mutation in ZNF674 gene. **The cases:** We describe two 48-old and 39-old brothers with non-syndromic mental retardation. Fragile X syndrome genetic testing and chromosomal microarray analysis were normal. Maternal examination was positive for skewed X-inactivation.

Results: Whole exome sequencing of the older brother revealed a novel hemizygous 46360713 A>G (Leu-104-Pro) mutation in ZNF674 gene. The variant was not found in large exome databases, and in-silico prediction programs classified the mutation as "pathogenic". According to sequence alignment of the ZNF674 protein from bacteria to human, Leu104 is a highly conserved residue throughout evolution. Family segregation analysis demonstrated the mutation in the younger brother with mental retardation, while two healthy males (a brother and a maternal uncle) were negative. The mother was found to be a heterozygous carrier.

Conclusion: Our research sheds light on the few controversial reports describing the relationship between ZNF674 gene mutations and intellectual disability, supporting this association.

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P08.44D

Cell cycle and neuronal differentiation defects in Kabuki-KMT2D mutant hESC and iPSC lines

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Introduction: Kabuki syndrome (KS) is a multi-systemic intellectual disability (ID) disorder. Loss-of-function (LoF) *KMT2D* mutations are responsible for a vast majority of cases of KS. *KMT2D* encodes a Histone3 Lysine4 methyltransferase but the underlying mechanism of ID in KS is unknown.

Materials and Methods: We generated (1) induced pluripotent stem cell (iPSC) lines from fibroblasts of patients with heterozygous LoF *KMT2D* mutations; and (2) a LoF heterozygous *KMT2D* in a wildtype human embryonic stem cell (hESC) line using the CRISPR-Cas9 approach. We differentiated the KS iPSC and hESC lines to form cortical neurons.

Results: In KS iPSC and hESCs, we observed significantly fewer cells (~10%) in the S-phase of the cell cycle when compared to control cells. Accordingly, in these cells we found a decrease in the expression of *CCDN1* and *E2F3*, two genes important for G1-S transition in the cell cycle. Additionally, we observed a delay in neuronal differentiation of KS iPSC and hESCs compared to control lines. In particular, at day 20 of the differentiation protocol, we observed a 4 fold decrease in formation of $CD44^{-}CD184^{-}CD24^{+}$ neuronal cells in KS mutant cells compared to control cells.

Conclusions: Our results suggest that correct dosage of KMT2D is essential for normal progression of cell cycle in pluripotent stem cells and that altered cell cycle may underlie delay in neuronal differentiation and ID in KS.

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P08.45A

New *KIAA1033* mutations in 3 patients with syndromic intellectual disability

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In 2011, *KIAA1033/SWIP* has been associated with autosomal recessive intellectual disability (AR-ID) in a large consanguineous family comprising seven affected individuals with moderate ID and short stature. Since that, no other case of *KIAA1033* mutation has been reported. Here, we report 3 patients from two unrelated couples with syndromic ID due to compound heterozygous *KIAA1033* mutations ascertained by whole exome sequencing (WES). Two sisters, aged 5.5 and 4 years, had a nonsense and a missense mutation inherited from their parents (p.Gln442* and p.Asp1048Gly), and presented with learning disabilities, macrocephaly, dysmorphic features, and skeletal features, associated with congenital absence of the right internal carotid with bilateral sensorineural hearing loss in the youngest. Our third patient was aged 32 years, had 2 missense mutations inherited from each parent (p.Lys1079Arg and p.His503Arg), and presented with mild ID, short stature and microcephaly. *KIAA1033* encodes a large protein named WASH4/SWIP which is part of the WASH complex. WASH complex is involved in the regulation of the fission of tubules that serve as transport intermediates during endosome sorting. Previous members of WASH complex *KIAA0196/WASHC5* have already been implicated in AR-ID with brain and cardiac malformations, under the designation of the Ritscher-Schinzel syndrome. WES has proved its efficiency to find replications of genes with insufficient data in the literature to be defined as a new OMIM gene. We conclude that *KIAA1033* is responsible of a non-recognizable AR-ID phenotype, and additional descriptions will be needed to refine the clinical phenotype.

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P08.47C

RNA sequencing and pathway analysis identify important pathways involved in hypertrichosis and intellectual disability in patients with Wiedemann-Steiner syndrome

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Growing number of histone modifiers are involved in human neurodevelopmental disorders, suggesting that proper regulation of chromatin state is essential for the development of the central nervous system. Among them, heterozygous *de novo* variants in *KMT2A*, a gene coding for histone methyltransferase, have been associated with Wiedemann-Steiner Syndrome (WSS), a rare developmental disorder mainly characterized by intellectual disability (ID) and hypertrichosis. As KMT2A is known to regulate the expression of multiple target genes through methylation of lysine 4 of histone 3 (H3K4me), we sought to investigate the transcriptomic consequences of *KMT2A* variants involved in WSS. Using fibroblasts from four WSS patients harboring loss-of-function *KMT2A* variants, we performed RNA sequencing and identified a number of genes for which transcription was altered in *KMT2A*-mutated cells compared to control ones. Strikingly, analysis of the pathways and biological functions significantly deregulated between patients with WSS and healthy individuals revealed a number of processes predicted to be altered that are relevant for hypertrichosis and intellectual disability, the cardinal signs of this disease. Network analysis of the present RNA-Seq data suggest that eNOS, WNT and BMP signaling pathways alterations may be linked to cognitive dysfunction and hypertrichosis in WSS.

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P08.48D

GENIDA, an international participative cohort study on genetic forms of intellectual disability and autism spectrum disorders: analyses of Koolen-deVries, Kleefstra, KBG and MECP2duplications syndromes

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Many recurrent CNVs and more than 700 genes are implicated in genetic forms of intellectual disability (ID) or autism spectrum disorders (ASD), but often with limited information on the clinical spectrum and natural history. We initiated cohorts study for genetic causes of ID/ASD, called GENIDA (https://genida.unistra.fr), whereby clinical information is entered by the family of the affected individual based on a clinical questionnaire (41 MCQ and 5 text qualitative questions, including adverse effect of drugs) that is currently available in 5 languages. We are internationally recruiting and have constituted a collection of cohorts (table1-top11). We have a questionnaire completion level above 85% for our best 300 participants. We have used Koolen-deVries syndrome to confirm that in general the data is in line with the literature. Importantly, we reported in detail growth parameters over time and the expected timing of delayed developmental milestones, including speech and language development, showed that social skills are relatively more preserved compared to other communication skills, and calculated - for the first time - the mean age of seizure onset (5.8 yo - n = 56). We are now extending our effort to other syndromes and will also present these results. This project shows the willingness and effectiveness of parents in participative studies. Direct comparison with published data allows us to search for novel and/or significant comorbidities and should promote better healthcare.

#	GENIDA cohorts - top11	Registered families	Active participants	Family access to analyses	Professionals of reference
1.	Koolen-deVries syndrome	223	188	All - except text	D. Koolen
2.	Kleefstra sydrnome	124	74	Overview / MCQ	T. Kleefstra
3.	KBG syndrome	55	42	Overview / MCQ	C. Ockeloen
4.	MECP2 duplication syndrome	47	30	Overview / MCQ	H. van Esch
5.	Valproate Neurodev. project	46	31	Under preparation	F. Francis & M. Nosten
6.	RASopathies	34	23	Overview only	A. Verloes & B. Kerr
7.	Cockayne syndrome	30	24	Overview only	N. Calmels
8.	22q11.2 duplication	19	13	Overview only	none yet
9.	MED13L	12	9	Overview only	none yet
10.	DYRK1A	9	6	Under preparation	A. Piton
11.	PCDH19	6	6	Overview only	none yet
...	...	...	...	...	...
	Total on GENIDA	860	535		

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P08.49A

A pseudogene increasing *LRFN5* expression in a patient with 14q21.2 deletion and autism

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Introduction: Autism spectrum disorder (ASD) is a disorder with impaired social relationships, language and communication, and is frequently associated with intellectual disability. Underlying genetic defects can be identified in 30-40% of ASD patients by chromosomal microarray analysis and whole exome sequencing.

Matherials and Methods: We report a 16 year-old boy with ASD bearing a microdeletion at chromosome 14q21.2 inherited from the father who has borderline cognitive impairment. The deletion affects a '*gene desert*' and *LRFN5* is the closest gene in the non-deleted interval. *LRFN5* encodes a protein involved in synaptic plasticity that has been implicated in neurodevelopmental phenotypes.

Results: We found decreased mRNA expression of both *LRFN5* gene and chr14.232.a pseudogene included within the deleted interval in the proband's fibroblasts compared to controls. We hypothesized the pseudogene chr14.232.a

regulates *LRFN5* expression. In agreement with this hypothesis, *LRFN5* expression was increased following transfection of the chr14.232.a pseudogene in the patient's fibroblasts.

Conclusion: The chr14.232.a pseudogene is predicted to bind miRNAs and based on the data generated so far, we speculate that the chr14.232.a pseudogene functions as a miRNA decoy to regulate *LRFN5* expression through sequestration of miRNAs targeting *LRFN5*. In conclusion, this study may unravel a novel mechanism of gene regulation involved in neurodevelopmental disorders.

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P08.50B

Three new cases of *MED13L* defect caused by a *de novo* novel frameshift mutations and a complex rearrangement

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Introduction: The *MED13L* gene was first associated with transposition of the great arteries and intellectual disability (ID). Later, it was recognised as distinctive syndromic ID phenotype presenting moderate to severe ID, facial anomalies, severe speech delay and muscular hypotonia in majority cases. At least 26 cases have been previously described.

Methods: We analysed retrospectively the results of 4813-gene panel and exome sequencing analyses (1495 cases) performed during 2014-2017 at Tartu University Hospital. In 25 unsolved exome sequencing trios, whole genome sequencing (WGS) was performed at Broad Institute. Copy number variant (CNV) calling from WGS data was performed using Manta software.

Results: We identified a novel *de novo* variant in *MED13L* gene in three cases (6 and 6.5-year-old females and 5.5-year-old male). Two patients had frameshift mutations - c.4245_4246del p.(His1415Glnfs*2) and c.5488_5495dup p.(Ser1833Ilefs*22). In the third case, CNV calling from WGS data revealed complex rearrangement with four breakpoints in *MED13L* gene. This patient has tandem duplication: chr12:116,661,676-116,668,880 and a deletion: chr12:116,662,161-116,675,475. The deletion disrupts exon 2 of *MED13L* gene presumably causing loss of gene function. All patients presented with mild to moderate ID, muscular hypotonia, ataxia or coordination problems and facial dysmorphism. Epilepsy, skeletal anomalies and strabismus were noticed once. No congenital heart anomalies were detected. One patient with complex CNV had severe speech defect.

Conclusion: We identified a pathogenic variant in *MED13L* gene in 0.2% of cases in our patient cohort. It makes *MED13L* one of the most common ID-associated genes among our diagnostic cohort. Funding: Estonian Research Council PUT355.

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P08.51C

***KCNT2*-related developmental and epileptic encephalopathy - a novel disease entity with potential for targeted treatment**

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Variants in several potassium channel genes have been found in developmental and epileptic encephalopathies (DEE). *KCNT1* and *KCNT2* belong to the SLO2 family of Na^+-dependent (K^+) channel genes, of which the latter has not been reliably associated with human disorders. We report two independent individuals with *de novo* variants in *KCNT2* at position R190. One of the two had West syndrome evolving to Lennox-Gastaut syndrome, the second individual had DEE with malignant migrating partial seizures of infancy. *In vitro* analysis suggested a gain-of-

function responsive to quinidine. A precision medicine approach in one of the two patients with add-on therapy of quinidine resulted in increase of alertness and vigilance, mild developmental progression, improved EEG and a temporary decrease of seizure frequency. We suggest that *KCNT2*-related disorders share similar phenotypic and *in vitro* functional and pharmacological features with *KCNT1*-related disorders and thus may represent a further example for disorders potentially responsive to targeted treatment opportunities.

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P08.52D

Molecular study of autism spectrum disorder discordant monozygotic twins indicates the role of *TRAP1* defects in disease susceptibility

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Introduction: Monozygotic twins (MZTs) have been considered to be physically and genetically identical. However, a significant number of disease-discordant MZTs, including pairs discordant for autism spectrum disorder (ASD), have been observed.

Materials and Methods: Three male MZT pairs discordant for ASD was recruited. DNA purified from hair follicles was used for whole exome sequencing (WES). For replication amplicon deep sequencing (ADS) was performed using DNA form MZTs' blood and hair follicles. Further validation was performed using 176 unrelated ADS patients.

Results: In one MZTs pair WES analysis revealed discordant missense variant in *RUVBL1* (p.Phe329Leu) and a nonsense variant in *TRAP1* (p.Gln639Ter). In autistic twin's hair follicles ADS confirmed the presence of *RUVBL1* variant with the mutant allele frequency (MAF) 42% and the *TRAP1* variant (MAF 8%); both variants were absent from hair follicles DNA of the healthy brother. However, in blood DNA both variants had the same MAF (*RUVBL1* 22%, *TRAP1* 2%) in both twins. Subsequent analysis of the whole coding sequences of *RUVBL1* and *TRAP1* genes in 176 unrelated ADS patients revealed no relevant variants in *RUVBL1*, whereas in *TRAP1* eight rare variants were identified, including the p.Gln639Ter.

Conclusions: Mutations in *TRAP1*, especially the p.Gln639Ter, may be associated with ADS. DNA purified from blood of disease discordant MZTs should not be used to perform molecular tests due to possible blood chimerism that may mask genetic differences.

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P08.53A

Confirmation of a recurrent mutation in NACC1 causing a severe neurodevelopmental disorder

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Only recently, the NACC1 gene was shown to harbor a recurrent mutation leading to a complex congenital neurodevelopmental disorder in 7 affected children (Schauch et al. 2017). All patients presented with a highly similar phenotype including severe intellectual disability (ID), developmental delay, cataract, microcephaly, severe infantile epilepsy, failure to thrive, irritability and stereotypic hand movements. Whole exome sequencing (WES) revealed the same de novo missense mutation in the NACC1 gene in all 7 children: c.892C>T p.(Arg298Trp). The NACC1 gene (nucleus accumbens associated protein 1) encodes a transcriptional repressor and has, until then, only been considered a candidate gene for ID due to its functional role and a missense mutation found by Gilissen et al., 2014 in a single patient with ID. Here, we describe a 5-year old girl with ID, microcephaly, cataract and movement abnormalities, leading to an initial diagnosis of a Rett-like phenotype in 2016. Panel sequencing of genes associated with Rett syndrome and an Array-CGH have been negative in our patient. Subsequently, using Trio-WES in the girl and her parents, the recurrent mutation c.892C>T in the NACC1 was found as a de novo mosaic and was the most likely disease-causing variant in this family. Comparison of the girls' clinical symptoms with those described by Schauch et al. showed a highly congruent phenotype. Our findings support the hypothesis that this NACC1 mutation causes a very specific phenotype.

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P08.55C

Identification of a novel de novo deletion of MAP3K8 suggestive of causing Noonan syndrome

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Noonan syndrome (NS) is a relatively common genetic disorder, characterized by distinctive facial features, short stature, congenital heart defect and developmental delay of variable degree. Known causative genes account for 70-80% of clinically diagnosed NS patients, but the genetic basis for the remaining cases is not known. The majority of the mutations identified in NS and other RASopathies are gain-of-function which result in increased RAS/MAPK signaling. However, previous studies have demonstrated that copy number variations, containing critical genes related to the RAS/MAPK signaling pathway, play a minor role in RASopathies. We describe a 7 year old girl that was clinically diagnosed with Noonan syndrome at the age of 10 months. Using exome sequencing, we recently identified a novel de novo ~3,2 Mbp deletion of multiple genes on chromosome 10p12.1p11.22, including MAP3K8. MAP3K8 is an oncogene, encoding a MAP kinase kinase kinase, that can phosphorylate and activate MAP2K1, which is associated with NS and CFC syndrome. We hypothesize that the 10p12.1p11.22 deletion leads to a dysregulation of the RAS/MAPK signaling pathway and therefore has an association with the phenotype of our patient. Functional analysis will be the next step to provide additional evidence that (micro)deletions containing MAP3K8 could result in NS.

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P08.56D

35 novel recessive candidate genes for intellectual disability and visual impairment by using 260 consanguineous families

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Consanguinity, practiced in a substantial fraction of human populations, reveals numerous rare recessive phenotypes because of the extensive regions of homozygosity by decent. The average total size of homozygosity is 253Mb in the offspring of consanguineous parents; this is 10fold higher than that of 25Mb in outbred individuals. Similarly, the rare homozygous variants (<2% frequency) in the coding regions are 57 in the offspring of first-cousins compared to 18 in outbred individuals. In Pakistan, the frequency of consanguineous marriages approaches 70%. We have initiated a Swiss-Pakistani project to identify novel recessive gene candidates for two phenotypes: intellectual disability (ID) and visual impairment (VI). We have collected samples from 145 ID and 205 VI families of first cousin marriages with at least 2 affecteds. Exome sequence of one affected and genotyping of the whole family (parents, all affected and unaffected siblings) has been completed in 114 ID and 146 VI families to date. The likely causative gene/variant in known genes was found in 67% of the VI and 34% in the ID families. Thus, there are more unknown recessive genes for ID. We have identified 21 novel candidate genes in VI and 14 in ID (to be presented in the conference). International matchmaking identified additional families in 20% of the candidate genes. Careful evaluation of the phenotypes is mandatory to assess the possibility of 2 or more causative genes, to eliminate false negative results. International databases from consanguineous individuals are needed to facilitate the assignment of pathogenicity to homozygous variants.

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P08.57A

Two novel splicing mutations in the OTUD6B gene associated with intellectual disability and seizures

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Introduction Biallelic mutations in the ovarian tumor domain-containing 6B (OTUD6B) gene, coding for a deubiquitinating enzyme, were recently described to cause an intellectual disability syndrome characterized by seizures and dysmorphic features in 6 families worldwide.

Materials and Methods We report on a 5-year-old Italian girl, presenting mild intellectual disability, speech and motor delay, and recurrent seizures. Whole-exome sequencing was performed on the proband DNA using the SureSelect Human All Exon V6 kit (Agilent) and the NextSeq500 instrument (Illumina). The identified mutations were confirmed by Sanger sequencing and their functional role was assessed by performing RT-PCR assays on the RNA extracted from peripheral blood mononuclear cells of the patient and her parents.

Results and Conclusions We identified two candidate heterozygous splicing mutations in the OTUD6B gene. These variants (c.324+1G>C and c.405+1G>A), both reported in the ExAC database with a frequency lower than the 1%, affect the donor splicing site of exon 2 and 3, respectively. Sanger sequencing confirmed the segregation of the variants in the family, showing that both parents are carriers of one mutation. RT-PCR experiments demonstrated that both variants affect OTUD6B splicing and lead to the production of aberrant transcripts, the major ones being, in both cases, the skipping of the upstream exon. Quantitative analysis performed by competitive-fluorescent RT-PCR on the patient RNA showed that the proband presents less than 1% of wild-type transcripts, further strengthening the causative role of these variants.

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P08.58B

A series of patients with *PHF6*-related disease: novel mutations and an expansion of the phenotype

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Börjeson-Forssman-Lehmann syndrome (BFLS, OMIM 301900) is a rare X-linked condition associated with intellectual disability, dysmorphic features and endocrine abnormalities. In recent years, a distinct phenotype, similar to Coffin-Siris syndrome, has been described in females with *de novo* mutations in *PHF6*. We ascertained 13 patients (8 males and 5 females) with *PHF6* mutations who were identified through clinical or research testing. Two families with 5 affected males had the same novel inframe deletion (p.E338del). Another male patient had a novel missense (p.D353H) which is the most distal pathogenic mutation yet described in *PHF6*. Female patients tend to have large *de novo* deletions, duplications or truncating mutations. Consistent features in the subjects included intellectual disability and the typical facial gestalt: broad nasal bridge, deep-set eyes, prominent or arched eyebrows, synophrys, brachydactyly, syndactyly, and large ears with fleshy lobes. Obesity, hormonal deficiencies and delayed puberty were common in male patients, whereas females often had linear skin hyperpigmentation, abnormal teeth and autistic traits. Unusual features in our series included umbilical hernias, keloid scarring, peri-ungal fibromas, absent vaginal orifice, lower limb motor neuropathy and talipes. The majority of our patients had squints or refractive errors. One had dysplastic optic discs while another had chorioretinal pigmentation and atrophy. One female patient with a novel missense mutation (p.G248V) had cortical abnormalities and poorly-controlled nocturnal frontal epilepsy. This is further evidence that females with BFLS are at risk of malformations of cortical development.

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P08.59C

Asymmetrical and Chemical-modified Donor-DNA Leads to Efficient Knock-ins of Pathogenic Mutations at CRISPR-Cas9-induced DNA Double Strand Breaks

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Introduction: Glycosylphosphatidylinositol (GPI) anchors attach a broad range of proteins to the cell membrane that have diverse roles in cell adhesion, signaling and complement regulation. Patients with a biosynthesis deficiency of the GPI anchor reveal global developmental delay, seizures and multiple malformations. Around 30 genes are involved in the molecular pathway and most pathogenic mutations have shown to be hypomorphic. However, the pathomechanisms on a cellular, tissue or organ level remain unclear and suitable animal models are lacking. We therefore chose to establish a mouse line for one of the most prevalent pathogenic missense mutations observed in humans so far *PIGV* c.1022C>A, p.Ala341Glu.

Methods: We co-transfected mouse embryonic stem (mES) cells with PX459-pSpCas9(BB)-2A-Puro (Addgene) vector and single-stranded oligodeoxynucleotides (ssODN) including the disease-causing mutation *PIGV* c.1022C>A. Positive clones were expanded and characterized by flow cytometry.

Results: We successfully integrated the pathogenic mutation *PIGV* c.1022C>A in mES cells using ssODNs with asymmetrical homology arms which were modified at the ends with phosphoro-thioat bonds. With this strategy, we were able to obtain up to 30% of knock-in (KI) clones. Furthermore, we observed that sgRNAs located directly at the missense mutation generated only homozygous clones. In contrast, we obtained heterozygous clones using sgRNA binding 13 bp upstream or downstream from the missense mutation. The generated *PIGV*-deficient mES cells were analyzed by flow cytometry and revealed a reduced surface expression of GPI-linked markers.

Conclusion: We here present a valid gene-editing strategy to generate efficiently KIs of missense mutations in mES cells via the CRISPR-Cas9 system.

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P08.60D

Pitt-Hopkins syndrome: dissecting the clinical and genetic heterogeneity of conditions in the phenotypic spectrum

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Pitt-Hopkins syndrome (PTHS, OMIM #610954) is a rare neurodevelopmental disorder caused by *TCF4* haploinsufficiency. Clinical features include severe intellectual disability, distinctive facial characteristics, breathing anomalies, postnatal microcephaly, and recurrent behavioral abnormalities. We studied 320 subjects referred to our Institute with a clinical suspicion of PTHS, who, in most cases, had already undergone several genetic tests with

normal results. We performed clinical evaluation and genetic analyses according to the following procedure: 1) detailed phenotype characterization by means of direct observation or the use of a specific questionnaire; 2) direct sequencing and MLPA of the *TCF4* gene; 3) NGS analysis of a panel of genes; 4) standard karyotype/ FISH analysis. Whole exome sequencing (WES) was performed in 16 patients. By clinical evaluation, a subset of 158/320 subjects was included in the PTHS spectrum. Among the 146 subjects analyzed by techniques 1) to 4), pathogenic variants were identified in 58 (40%), involving the following genes: *TCF4* (tot:42), *UBE3A* (tot:4), *MECP2* (tot:6), *FOXG1* (tot:3), *ZEB2* (tot:2), *ATRX* (tot:1). Further causative variants were identified by WES in 4/12 analyzed patients, in *EHMT1*, *SZT2*, *ASXL3* and *GABRB2*. Through an in-depth molecular characterization of a further *TCF4* variant non associated with a classical PTHS phenotype, and a critical review of other similar cases in scientific literature, we suggest an intragenic phenotypical map of *TCF4*, reflecting the selective disruption of different functional domains of the protein.

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P08.61A

Intellectual disability due to a *PPP2R5D*-mutation; what do we know about natural history and adult phenotype?

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PPP2R5D is a gene associated with neurodevelopmental disorders and intellectual disability. The phenotype includes large ventricles, macrocephaly, hydrocephalus and (partial) corpus callosum agenesis. Data on the natural history in adulthood is limited.

We describe a female, 42 years, with a severe intellectual disability, macrocephaly, corpus callosum agenesis, open vertebral bow and no seizures. Trio exome sequencing resulted in identification of a de novo p.Glu198Lys mutation in the *PPP2R5D*-gene. This mutation has been previously described and is associated with the most severe phenotype. Interestingly, in our patient profound myopia, strabismus convergens fixus and retinal punched out lesions were identified. To our knowledge, no retinal abnormalities were associated with mutations in the *PPP2R5D*-gene until now.

Punched out lesions are associated with inflammation of the retina and choroidea, described in posterior uveitis as well as after congenital infection. There was no history of uveitis or congenital infection in our patient. Interestingly, such lesions have been described in Aicardi syndrome. Aicardi syndrome is a neurodevelopmental disorder characterized by infantile spasms, agenesis of the corpus callosum, and retinal abnormalities. The cause of the disease remains unclear.

We see some overlap in the phenotype of *PPP2R5D*-mutations and Aicardi syndrome. Mutations in *PPP2R5D*-gene have not been previously identified in Aicardi cohort.

This case illustrates the clinical phenotype and natural history of a *PPP2R5D*-mutation in an adult patient. We recommend to perform ophthalmological examination in patients with *PPP2R5D*-mutation to identify visual impairment and to find out whether the retinal abnormalities are part of the *PPP2R5D*-phenotype.

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P08.62B

Dysmorphic phenotype in patients with RAB39B mutation

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Background: Mutations in the gene *RAB39B* (Xq28) have been associated with X-linked mental retardation-72 (#MIM300271, including intellectual disability (ID) and autism spectrum disorders (ASD)), and Waisman syndrome (#MIM311510), characterized by ID and early-onset Parkinsonism. RAB39B regulates GluA2 trafficking to determine synaptic AMPAR composition. In this report we describe a family with two affected males with severe ID and characteristic dysmorphic features not previously reported in patients with *RAB39B* mutation.

Methods: Clinical phenotyping, MLPA and whole exome sequencing (WES) combined with targeted analysis focused on 119 genes related to X-linked ID was performed on four members of this family.

Results: The proband case, a 18-year-old male, presented with ID, ASD, epilepsy and behavioral and sleep disorders. The maternal uncle was a 57-year-old male with severe ID. Both showed long and hypomimic face, bilateral ptosis, lower lid ectropion, thick and everted lower lip, protruding ears and drooling and swallowing difficulties, unlike other family members. Two novel variants were identified in *RAB39B* and *HCFC1* in the proband, the mother and the maternal uncle, but not in the brother. The first one was a hemizygous mutation in *RAB39B* (NM_171998.3:c.137dup, p.Ser47Leufs*44) with a likely pathogenic significance. The second one was a hemizygous nonsense mutation in *HCFC1* (NM_005334.2:c.2984C>G, p.Thr995Ser), predicted to be probably tolerated. These genes are not known to interact with each other (dSysMap). X-chromosome inactivation in the mother was not skewed.

Conclusions: In addition to ID and ASD, *RAB39B* mutations could be associated with a characteristic dysmorphic phenotype.

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P08.63C

RAC1 missense mutations cause diverse phenotypes and define Rhopathies as a new group of developmental disorders

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Introduction: RAC1 is a highly conserved Rho GTPase under strict mutational constraint.

Methods and Results: We report seven individuals with intellectual disability and distinct *de novo* missense *RAC1* mutations. These included four patients with microcephaly (OFC between -2.5 to -5 SD; p.Cys18Tyr, p.Asn39Ser, p.Pro73Leu and p.Cys157Tyr), two with macrocephaly (OFC +4.16 and +4.5 SD; p.Val51Met and p.Val51Leu) and one individual with normal OFC (p.Tyr64Asp).

In silico modeling, mouse fibroblasts spreading assays and *in vivo* overexpression assays using zebrafish as a surrogate model demonstrated that (a) the p.Cys18Tyr and p.Asn39Ser *v*ariants are dominant-negative alleles and result in reduced neuronal proliferation, microcephaly and cerebellar abnormalities; (b) the p.Tyr64Asp variant is constitutively active; and (c) the effect of other variants is likely context dependent.

RNAi-mediated knockdown of *CYFIP* homologue, *sara1*, in drosophila neurons with constitutively active p.Tyr64Asp *rac1* variant resulted increased the rate of embryonic lethality.

Conclusions: *RAC1* missense mutations orchestrate diverse human phenotypes with an extraordinary spread of ~10 SD of head circumferences. These findings highlight the importance of RAC1 in neuronal development and demonstrate the complexity of defining novel rare diseases with extreme phenotypic variability.

Mutations in several genes encoding components or regulators of the Rho signaling pathway (e.g. *CDC42, ARHGAP31, TRIO, HACE1, ELMO2, DOCK6* and *SMPX*) have been identified in human disorders recently. We propose that this emerging sub-category of rare developmental disorders to be designated as Rhopathies with RAC1 as its central player. Our and others' work indicate that some Rhopathies may be amenable to treatment.

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P08.64D

Whole genome sequencing of consanguineous families reveals novel pathogenic variants in intellectual disability

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Intellectual disability (ID) is a common disorder affecting more than 2% of the population, but the majority of patients receive no molecular diagnosis. The disorder is highly genetically heterogeneous, with estimates of more than 2,500 autosomal ID genes. Autosomal recessive ID genes have primarily been identified by studies of patients from consanguineous families. Here we used whole genome sequencing to study six consanguineous families that had previously been tested using whole exome sequencing without obtaining a molecular diagnosis. For four of the six families (66%) we identified pathogenic variants in genes previously reported in patients with ID (*PIGN, SMC1A, FRRS1L*, and *RTTN*). Additionally, in one patient we identified variants in both *FRMD4A1* and *COL27A1*. The *COL27A1* variant explains the skeletal malformations in the patient, but also expands the phenotype of this gene to involve hearing impairment and ID. Our study highlights the benefits of whole genome sequencing in families where diagnostic exome sequencing was unsuccessful. Our results provide new pathogenic variants in several autosomal recessive ID genes and describe a second finding of pathogenic variation in *COL27A1* in a patient with both skeletal malformations and ID.

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P08.65A

Quantifying the contribution of recessive coding variation to developmental disorders

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Large scale sequencing is illuminating the genetic architecture of rare diseases. We analyzed 7,447 exome-sequenced families from the Deciphering Developmental Disorders study, and estimated the genome-wide contribution of recessive coding variation in both known and as-yet-undiscovered genes. Our approach is the first to allow a properly calibrated estimate of recessive burden. We found that the proportion of cases attributable to recessive coding variants was only 3.6% in patients of European ancestry, compared to 50% explained by *de novo* coding mutations. It was higher (31%) in patients with Pakistani ancestry, due to elevated autozygosity. Half of this recessive burden is attributable to known genes. Three genes were significantly enriched for biallelic variants after stringent Bonferroni correction. One is a new disease gene (*EIF3F*), and another, *KDM5B*, is already reported in association with dominant disease. The signal in *EIF3F* ($p = 1.2\times10^{-10}$) is driven by a single missense variant (frequency ~0.1%) that was homozygous in 9 DDD probands, and we are currently testing its effect in cell lines. *KDM5B* ($p = 1.1\times10^{-7}$) appears to follow a complex mode of inheritance, in which heterozygous loss-of-function (LoF) variants show incomplete penetrance and homozygous LoFs are fully penetrant. Through simulations, we estimate the number of as-yet-undiscovered genes that act by a recessive coding mechanism. Our results suggest that recessive coding variants only account for a small

fraction of currently undiagnosed individuals, and that future studies should focus on noncoding variants and polygenic mechanisms.

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P08.66B

The functional consequences of *SCN2A* mutations determine the phenotype

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Objective: Mutations in the voltage-gated sodium channel type 2 ($Na_v1.2$) lead to a broad spectrum of phenotypes ranging from self-limited familial neonatal-infantile epilepsy (BFNIE) to very severe epileptic encephalopathy (EE) to intellectual disability without seizures (ID), yet the underlying mechanisms determining this phenotypic variability are incompletely understood. In this study, we therefore investigate the functional effects of six mutations to elucidate the different pathomechanisms leading to EE or ID.

Methods: Six pathogenic *SCN2A* mutations underlying either EE, ID or BFNIE were selected for functional studies. Biophysical properties of recombinant wildtype and mutant $Na_v1.2$ channels were measured using voltage-clamp of transiently transfected HEK293T cells co-expressing auxiliary β-subunits and EGFP. In-silico protein modeling was used to gain insight into the structural effects of the mutations.

Results: Both *SCN2A* missense mutations causing EE showed profound gating changes in patch-clamp experiments, whereas all ID mutations, nonsense and missense, exhibited no relevant current. The BFNIE mutation showed a small change of channel inactivation resulting in a small gain of function. The protein modeling suggested structural aberrations for all studied missense mutations consistent with the electrophysiological findings.

Discussion: By examining the functional consequences of *SCN2A* mutations causing epileptic encephalopathy, self-limited familial neonatal-infantile epilepsy and intellectual disability this study contributes to the elucidation of mechanisms leading to the broad phenotype variability reported for *SCN2A* mutations. Our findings support the hypothesis that complete loss-of-function mutations lead to intellectual disability without seizures, small gain-of-function mutations cause BFNIE and epileptic encephalopathy mutations exhibit variable but profound $Na_v1.2$ gating changes.

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P08.67C

A recurrent *de novo* missense mutation in *SMARCB1* causes severe intellectual disability and choroid plexus hyperplasia with resultant hydrocephalus

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Introduction: *SMARCB1* encodes a subunit of the SWI/SNF-complex involved in chromatin remodeling. Mutations in this gene can give rise to three conditions. Heterozygous loss of function germline mutations cause the rhabdoid tumor predisposition syndrome and schwannomatosis. Presumed gain of function missense mutations in exon 8 and 9 result in Coffin Siris syndrome, which is characterized by intellectual disability (ID), coarse facial features and fifth digit anomalies.

Methods: By a gene matching approach, individuals with a similar *SMARCB1* mutation were identified. Informed consent was obtained and patient data were collected to further establish genotype-phenotype relationship.

Results: A recurrent *de novo* missense mutation (c.110G>A;p.Arg37His) in exon 2 of *SMARCB1*, encoding the DNA-binding domain, was identified in four individuals from different genetic centers. They shared a distinct phenotype consisting of profound ID and hydrocephalus due to choroid plexus hyperplasia. Other shared features include severe neonatal feeding difficulties, congenital heart-, kidney-, and eye anomalies, obstructive sleep apnea and anemia.

Conclusion: The p.Arg37His mutation in the DNA binding domain of *SMARCB1* causes a distinctive syndrome, probably through a gain of function, which is characterized by severe ID and hydrocephalus resulting from choroid plexus hyperplasia. This report broadens the phenotypic spectrum associated with mutations in *SMARCB1*.

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P08.68D

RAI1 intragenic deletion and concomitant overexpression in a syndromic patient: Smith-Magenis or Potocki-Lupski syndrome?

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Smith-Magenis Syndrome (SMS) [MIM:182290] is a genomic disorder caused by *RAI1* gene haploinsufficiency and characterized by intellectual disability, craniofacial dysmorphisms, behavioral and sleep disturbances, speech and motor delay. Here we describe a 17 years old girl with a clinical suspicion of SMS. Upon previous exclusion of 17p11.2 SMS locus deletion, *RAI1* mutational screening identified the yet unreported heterozygous variant p.A1091D predicted as likely benign and inherited from the healthy mother. Moreover, MLPA analysis revealed a *de novo* heterozygous deletion encompassing *RAI1* exon 5, that encodes PHD functional domain, consistent with the initial clinical suspicion.In order to verify that the *de novo* deletion results in *RAI1* haploinsufficiency, RT-qPCR studies were carried out but showed an unexpected significant increase in blood transcript levels of both patient and mother compared to those of 10 controls. Moreover, a specific allelic dosage analysis revealed that the deleted allele is overexpressed in the patient and concerns the inherited maternal allele. This result confirms SMS diagnosis, as an overexpression of an aberrant transcript lacking the functional domain was detected.Our finding supports the hypothesis that *RAI1* overexpression, shared with the mother, can be mediated by a *cis* element. Promoter and regulatory regions are under study aiming at identifying variants that could be related with *RAI1* overexpression finding. Despite *RAI1* overexpression causes Potocki-Lupski Syndrome (PTLS) [MIM:610883], the mother does not resemble a PTLS clinical phenotype. This might be explained by the PTLS high phenotype variability or by penetrance defect as previously reported in other familiar cases.

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P08.69A

Maternal transmission of mild Coffin-Siris syndrome phenotype due to a _SOX11_ missense mutation

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Since 2014, mutations in *SOX11* are known to cause mild Coffin-Siris syndrome (CSS) phenotype. SOX11 is a

transcription factor of the PAX6-BAF complex, which is proposed to play a role in brain development.

Here we report for the first time of a maternal transmission of Coffin-Siris syndrome (CSS) due to a *SOX11* missense mutation. We present two daughters (14 and 10 years of age) of non-consanguineous parents with intellectual disability and muscular hypotonia. Both sisters showed mild dysmorphic facial features such as short philtrum, thick lips, low-set ears and strabism. Cogan ocular motor apraxia was present in both sisters. Mother and both daughters showed hypoplastic nails of the fifth toes as sign of mild CSS. The mother also had a history of learning difficulties. A coloboma of the iris was present.

Karyotyping and array-CGH gave normal results. NGS panel diagnostics for CSS revealed a missense variant in *SOX11* [c.139G>A; p.(Gly47Ser)] in both sisters and their mother. Four *in silico*-tools (PROVEAN, SIFT, Polyphen-2 and MutationTaster) predicted the mutation as probably pathogenic.

A review of the literature showed that until now only six patients with de novo mutations in SOX11 have been described. All of them showed intellectual disability and hypoplastic nails of the fifth toes. Some of these patients had Cogan ocular motor apraxia. Facial dysmorphic features seem not to be specific. We suggest that the combination of Cogan ocular motor apraxia, developmental delay and hypoplastic nails of fifth toes are important diagnostic criteria for recognizing patients for mutations in *SOX11*.

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P08.70B

Novel ST3GAL3 mutations in three patients with intellectual disability and epilepsy

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The *ST3GAL3* gene located on chr1p34.1 encodes for the β-galactosidase-α2,3 sialyltransferase-III (ST3Gal-III), a glycoprotein/ganglioside sialylytransferase present in Golgi membrane and playing a role in interactions of brain cells with their environment thought glycocalyx. Pathogenic variants in *ST3GAL3* were reported in three consanguineous families only in patients with nonsydromic intellectual disability or with epileptic encephalopathy (West syndrome) and severe developmental delay. We report three unrelated patients (two brothers and a sporadic case) to further delineate the phenotype associated with *ST3GAL3* mutations and describe with more details the phenotype of previously reported patients. All of our three novel patients had early epilepsy and severe to profound intellectual disability. The epilepsy was transient in one and pharmacoresistant in the siblings with a more severe neurodevelopmental phenotype. We identifed by whole exome sequencing and with a sequencing gene panel two novel homozygous variants in *ST3GAL3*: the c.765-1G>A intronic variant in one patient and the c.1015C>T (p.Arg339*) nonsense mutation in the brothers. Our three patients and those of the literature share severe neurodevelopmental delay without language acquisition. Epilepsy is constant and variable ranging from few seizures to pharmacoresistant epileptic encephalopathy. The siblings had the most severe phenotype reported to date and microcephaly, a feature not previously reported in the condition. MRI, EEG and the patients' morphology are non specific. We report the first truncating mutation and the first splice site mutation predicted to product exon skipping, which may explain that the patient has a less severe phenotype.

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P08.71C

Alterations to synaptic vesicles pathways are likely to be involved in non-syndromic intellectual disability

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Introduction. Pathway prioritization is a challenging yet promising tool for uncovering mechanisms and therapy targets for intellectual disability (ID). Using big genomic data and advanced *in silico* techniques, it is possible to unmask pathways and processes, alterations to which can be causative to neurobehavioral diseases.

Materials and Methods. To reveal candidate processes for neurobehavioral problems, we used high-resolution genome-wide CNV scan in 191 children with congenital abnormalities and idiopathic ID (Affymetrix CytoScan HD) and in 11 children with Rett syndrome-like phenotype without *MECP2* mutations (Nimblegen 12×135K). All children were not exhibiting cytogenetically visible chromosomal abnormalities and detectable genomic rearrangements and epigenetic changes. A bioinformatic algorithm (Iourov et al., 2014) was applied for candidate pathway prioritization using KEGG, Reactome, Gene Ontology, NCBI biosystems databases.

Results. Pathway prioritization yielded enrichment of 4 pathway clusters in both cohorts: vesicles functioning, Notch signaling pathway, actin functioning, transcription regulation. Among these clusters, the SNAP receptor activity pathway (GO:0005484) was found to be the most significantly prioritized. Synaptic vesicles are mostly located at presynaptic terminal and carry neuromediators. Alterations to vesicles docking and exocytosis can affect neurotransmitter release leading to neurobehavioral diseases.

Conclusions. Since synaptic vesicles fusion with presynaptic plasma membrane is essential for nerve impulse transmission, these data do not only hallmark alterations to synaptic vesicles pathways involved in non-syndromic intellectual disability, but also indicates possible therapeutic targets for molecular interventions. Finally, it seems that synaptic vesicles pathways represent an intriguing target for further studies of ID pathogenesis. Supported by RSF (14-35-00060).

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P08.72D

Effect of modifier genes within Copy Number Variations in chromosome 16p on the body weight of intellectual disability patients

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Introduction: Syndromic patients could manifest intellectual disability associated to early-onset weight alteration (underweight and/or obesity). The diverse heterogeneity in its etiology has improved the development of techniques for genetic diagnosis, allowing the molecular characterization of new syndromic phenotypes. The use of genetic databases of patients with intellectual disability and other described clinical traits is evidencing correlations with specific molecular alterations or combination of them.

Patients and Methods: We present an exhaustive description of Copy Number Variants (CNVs) identified in chromosome 16p from 191 CytoScan HD Affymetrix arrays performed in syndromic patients and their relationship with severe alterations in corporal and body weight composition.

Results: Variants were localized in chromosome 16p and were classified as: (I) previously non-described 10 Mb duplication in the 16p13.2p12.3 region considered causal of the developed phenotype which consisted on intellectual disability and obesity, and (II) CNVs localized in the 16p11.2 region characterized by their low prevalence but with recurrence in syndromic patients with severe alterations in the corporal weight. Proximal 16p11.2 CNVs had a dose-dependent effect: underweight in case of duplication and obesity in case of deletion. Our analysis has allowed suggesting *KCTD13* gene as candidate to produce the physiopathology of the 16p11.2 proximal syndromes and *SH2B1* gene for the 16p11.2 distal phenotypes.

Conclusion: CNVs of chromosome 16 postulate it as genomic hotspot of alterations in the body mass index in syndromic patients, allowing the primary prevention of comorbidities with its detection. Studies in syndromic individuals could constitute a reliable model to evaluate hypothalamic satiety disorders.

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P08.73A

Taurine administration recovers motor and learning deficits in an Angelman syndrome mouse model

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Angelman syndrome (AS, MIM 105830) is a rare neurodevelopmental disorder affecting 1:10-20000 children. Patients show moderate to severe intellectual disability, ataxia and absence of speech. Currently no treatment is available. Studies on both post-mortem AS human brains and mouse models revealed dysfunctions in the extra synaptic GABA receptors implicated in the pathogenesis. Our study focused on taurine, a free intracellular aminoacid, abundant in brain and considered an inhibitor neurotransmitter with neuroprotective properties. As taurine acts as agonist of GABA-A receptors, expressing various GABA-A subunits, we aimed to investigate if it might ameliorate AS symptoms. Since the mice weaning, we orally and chronically administered 1 g/kg taurine in water to *Ube3a* deficient mice. In order to test the improvement of motor and cognitive skills, rotarod, NORT and Open Field tests were assayed at 7, 14, 21 and 30 weeks, while biochemical tests and aminoacid dosages were carried on respectively by western blot and HPLC on frozen brains. An increased level of GFAP and an activation of ERK1/2 pathway were observed in the total brain of transgenic mice. Taurine treatment significantly rescued motor and learning skills and restored the level of the glial marker GFAP and of pERK1-2/ERK1-2 ratio. Our study indicates oral taurine administration as a potential therapy to ameliorate motor deficits and learning difficulties in AS.

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P08.74B

Scientific yield of clinical exome sequencing of neurodevelopmental disorders

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Introduction: We present our results of scientific evaluation of clinical trio exome sequencing of neurodevelopmental disorders (NDD).

Materials and Methods: We performed trio exome sequencing of 203 undiagnosed NDD cases. Of these, 59% were preceded by a large, but negative diagnostic panel. Sequencing was performed by Centogene or CeGaT using well-established enrichment kits and sequencing platforms. Bioinformatic analyses were performed by Limbus. If exome sequencing did not reveal a clear causative variant in an established NDD gene, we searched for candidate genes on scientific basis. To estimate the relevance of candidate genes, we established a scoring system using 13 parameters, based on inheritance, gene and variant attributes, and published literature.

Results: A reliable clinical genetic diagnosis was made in 60 (29.5%) trios, corresponding to 20% in cases preceded by panel diagnostics and 43% in cases without prescreening. In the remaining 143 (70.5%) trios, re-evaluation revealed potentially causative variants in 146 candidate genes. We applied our scoring system to prioritize the relevance of these variants. The scores varied between 2 and 11.9. The top 10% of the scores (score > 9) are *ASIC1, TANC2, FBXL19, KMT2E, GLS, ACTL6B, GRIN3B, CUX1,*

UNC13A, GRIA4, PUM2, TOP1, WDFY3, NPTX1 and *SPEN*.

Conclusion: For the majority of the candidates with highest scoring variants additional cases have been identified through our network of collaborators. This confirms disease associations and enables spin-off studies. Our results illustrate the enormous scientific value of the re-evaluation of the substantial amount of negative exome sequencing samples.

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P08.75C

Genotype-phenotype correlation associated with *de novo* missense variants in *TRRAP*: from autism spectrum disorder to syndromic intellectual disability

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Acetylation of histone lysine residues is a major component of transcriptional regulation. This tightly regulated reaction is performed by histone acetyltransferases (HATs). The recruitment and activity of HATs depend on a multiprotein complex, that includes cofactors and a large scaffolding protein of 3859 amino acids called TRansformation/tRanscription domain-Associated Protein (*TRRAP*). Through an international collaboration and by use of GeneMatcher, 15 *de novo* variants in *TRRAP* (NM_001244580.1) were identified from research and clinical exome sequencing cohorts, in 21 patients from 20 families (1 germinal mosaicism). All variants were absent from gnomAD. Remarkably, a strong genotype to phenotype correlation was observed with two clinical spectra. The first is a complex, multi-systemic syndrome characterized by severe intellectual disability (ID) in addition to various malformations of the brain, heart, kidneys and genitourinary system. Patients with this phenotype carried missense variants (I1031M, R1035Q, S1037R, E1104G, E1106K, G1111W, G1159R) that clustered around a substitution identified in 5 individuals (A1043T). The second presentation includes individuals with autism spectrum disorder and/or ID and epilepsy, with another cluster of variants including R1859C, W1866C, W1866R, G1883R and P1932L as well as non-clustering variants (L805F and F860L). *TRRAP* is highly conserved evolutionarily and is among the top five genes intolerant to missense variations. RNAseq on patient cell lines identified several hundred misregulated genes; with an enrichment in genes involved in neuronal development and axonal guidance, developmental processes, cell-cell adhesion and motility. qRT-PCR, ATACseq and Immuno-histochemistry experiments are ongoing to confirm the results and gain further insight into pathophysiology.

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P08.76D

Analysis of whole exome sequencing data with a panel of genes associated with intellectual disability and epilepsy in a diagnostic lab

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Introduction: The implementation of whole exome sequencing (WES) in the clinic has rapidly increased the diagnostic yield in patients with (syndromic) intellectual disability (ID) and/or epilepsy. Here we present an overview of the first 109 patients that were analyzed with our accredited ID & Epilepsy panel, a WES based targeted panel analysis.

Material and Methods: gDNA was enriched for (coding) exons with the Sureselect All Exon v6 kit (Agilent Technologies) followed by paired-end 2x150bp sequencing on a HiSeq3000 platform (Illumina). Raw sequence reads were processed using an in-house developed pipeline (Seqplorer) and data analysis was limited to a panel of 1109 selected Intellectual Disability & Epilepsy genes.

Results: We've found a possible molecular explanation for the aberrant phenotype of the patient in 23% of the cases. The majority of the (likely) pathogenic variants arose de novo (71%), few cases could be explained by variants in genes related to respectively X-linked recessive disorders (17%) or autosomal recessive disorders (8%). Several interesting findings were observed, for example, we've identified de novo SATB2 missense variants in two unrelated patients affecting the same codon but different nucleotide.

Conclusion: To conclude, since the introduction of whole exome sequencing in the Center for Medical Genetics Ghent, we were able to provide a possible molecular diagnosis (that could explain the clinical features) for a high percentage (23%) of patients. This is in concordance with previous reports in literature with a diagnostic yield ranging from 16-29.4% (de Ligt, 2012 and Monroe GR, 2016).

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P08.77A

The phenotypic spectrum of WWOX-related Epileptic Encephalopathy: 20 additional cases and review of the literature

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Germline bi-allelic mutations in *WWOX* have been associated with spinocerebellar ataxia type 12 (SCAR12) and WWOX-Related-Epileptic-Encephalopathy (WOREE syndrome). We report on 20 additional patients with WOREE syndrome.

SNVs and CNVs were identified by means of pangenomic approaches (WES and aCGH) and/or *WWOX* targeted molecular screening. All missenses identified (included eight novel mutations) are classified as pathogenic or likely pathogenic according to the ACMG recommendations. The phenotype of our patients was consistent with previously reported cases. All individuals had severe developmental delay (inability to walk and no speech development) and early onset epilepsy. In contrast to previous reports, growth retardation (6%) and microcephaly (20%) were not prominent signs in our series. The most striking dysmorphic features were a round hypotonic face with full cheeks and a short neck. Additional medical problems were visual impairment (75%), spine deformity (65%), feeding (70%) and respiratory (40%) problems. Brain MRI was abnormal in 80% of patients showing corpus callosum hypoplasia (75%) and progressive cerebral atrophy (55%). By aggregating our patients with all cases reported so far, there are currently 37 patients with a WOREE syndrome. No clear genotype-phenotype correlation is emerging. It was initially claimed that homozygous or compound heterozygous missense(s) genotypes could lead to a SCAR12 phenotype. In our cohort, we describe 5 patients with missense(s) genotypes and a WOREE syndrome. The most severe clinical presentation seems to be associated with genotypes corresponding to virtual *WWOX* full knock-down.

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P08.78B

Ubiquitin-proteasome system impairment and intellectual disability: the *CUL4B* example

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Introduction: Pathogenic variants in the *CUL4B* gene are responsible for the clinical phenotype of *CUL4B*-related X-linked intellectual disability (ID) or Cabezas syndrome (MIM #300354). *CUL4B* encodes a scaffold protein, the cullin-4B which is enrolled in the Cullin4B-RING ubiquitin ligase (E3) complex. This complex plays an essential role in the recognition and ubiquitination of proteins which occurs prior to their cellular degradation by the 26S proteasome.

Materials and Methods: Through an international multicentric investigation based on exome sequencing, we identified 15 male individuals with unpublished *CUL4B* pathogenic variants. In order to determine if the ubiquitination/protein system (UPS) was affected by these *CUL4B* alterations, we performed protein analysis by native SDS-PAGE and western-blot on peripheral blood mononuclear cells (PBMCs) from subjects and healthy controls.

Results: We identified 9 frameshift indels, 1 in-frame indel; 4 nonsense and 1 missense variants. All affected individuals presented with mild to severe ID with speech and motor delay, along with more variable neurological, skeletal and dysmorphic features. Interestingly, the 20S and 26S proteasome complexes of the tested individuals exhibited a higher chymotrypsin-like activity than those of the controls. This finding was confirmed by measuring the degradation rate of the Suc-LLVY-AMC substrate in whole-cell extracts.

Conclusions: Our initial results suggest a direct implication of CUL4B in the regulating function of proteasome 26S. CUL4B could indirectly contribute to the maintenance of cellular protein homeostasis. However, the mechanisms through which these interactions are exercised and their consequences, particularly in the pathological context of intellectual impairment, still remain to be clarified.

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P09 Neurogenetic and psychiatric disorders

P09.001A

Dissecting tissue-specific functional networks associated with 16p11.2 reciprocal genomic disorder using CRISPR engineered human iPS and mouse models

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Reciprocal genomic disorders (RGDs) represent a recurrent class of copy number variants (CNVs) that collectively comprise a major contributor to neurodevelopmental disorders (NDD) and altered anthropometric traits. Here, we systematically dissected the functional networks associated with 16p11.2 RGD from transcriptome analyses of 70 mice with reciprocal CNV of the syntenic 7qF3 region across

cortex, striatum, and cerebellum, as well as liver, white and brown adipose tissues in a subset of 16 mice (n = 250 samples). We integrated these data with brain tissues from a *Kctd13* mouse model (a putative driver of 16p11.2 neuroanatomical phenotypes, n = 50), and CRISPR-engineered, isogenic 16p11.2 iPSC-derived NSCs (n = 25) and induced neurons (n = 27). The strongest magnitude of effect sizes from 7qF3 were observed across brain regions by comparison to non-brain tissues (cortex 7qF3 region average p-value = 8.80E-35; non-brain p = 0.0013), reflecting the ~3x higher basal expression changes. Coexpression network analyses isolated a consistent module of 16p11.2 genes, as well as a module that was highly enriched for constrained genes (ExAC pLI≥0.9), autism-associated genes, early fetal development coexpression networks derived from BrainSpan, and neurological phenotypes and processes. Differentially expressed genes (DEGs) were enriched in this 'constrained' module network; moreover, DEGs from the Kctd13 mouse coalesced into this same module (cortex enrichment p = 7.82E-41), suggesting overlap in altered transcriptional networks between full length CNV and deletion of KCTD13 alone. These analyses identify a tissue-specific impact of 16p11.2 RGD that converges on a module of co-expressed genes that are intolerant to genetic perturbation and associated with critical processes in human neurodevelopment.

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P09.003C

Molecular characterization of a novel *RNASEH2B* splice site mutation responsible for Aicardi-Goutieres syndrome

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Aicardi-Goutières syndrome (AGS) is a genetically determined inflammatory encephalopathy characterized by a resemblance to congenital infection. Early onset in childhood is often observed, but variability in presentation and course is recognized, encompassing peripheral spasticity to severe psychomotor disability. To date, mutations in seven genes have been described to cause AGS. *RNASEH2B* gene (MIM# 610326), encoding the RibonucleaseH2 beta subunit, is most frequently mutated, seen in association with ~40% of diagnosed patients. Here we report a new case of AGS in a 4 year old girl who, after an infectious gastroenteritis, developed a left spastic hemiparesis with predominant dystonia of the upper limb, in association with white matter abnormalities on brain MRI. Biochemical investigation revealed high neopterin and slightly elevated interferon (IFN)-α levels in the cerebrospinal fluid, with a variable elevation of IFN-regulated-gene transcripts in the peripheral blood. By targeted next generation sequencing performed on genomic DNA, we identified a hitherto unreported *RNASEH2B* intronic variant, c.699-9C>G, in the compound heterozygous state with the recurrent mutation c.529G>A (p.Ala177Thr). Sanger sequencing confirmed biallelic segregation consistent with autosomal recessive inheritance. The study of *RNASEH2B* transcript expression in leucocytes by RT-PCR in the proband, and her healthy heterozygous carrier mother, demonstrated that the novel variant creates a new 3' acceptor splice site upstream of the boundary of exon 9, inducing the retention of an intronic fragment in the mature mRNA, with predicted loss of the STOP-codon. An investigation of the impact of this variant on *RNASEH2B* transcript stability and translated protein is in progress.

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P09.004D

A whole exome study of Alzheimer's Diseases which is augmented by population data found the noble AD risk genes

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Alzheimer disease (AD) has high heritability. The AD Sequencing Project (ADSP) which includes 10,909 participants, aimed to find additional AD risk genes harboring low frequency and rare coding variants. However, ADSP may be still underpowered for very rare alleles. In this study, we augmented statistical power by using the ExAC database as comparison group for the ADSP cases and tried the noble AD risk and protective genes. In order to avoid the population bias, we included only Non-Finnish European population in ExAC. In ADSP, we used European ancestry and excluded outliers by the plot of the first two principal components. We selected variants with minor allele frequencies (MAF) between that of the ADSP cases and ADSP controls. The MAF differences were tested by Pearson's chi-square tests. The MAF of gene-based analysis were obtained by collapsing MAFs of variants within gene boundaries. In the variant level, the well-known AD genes-TOMM40, TREM2, and MS4A6A-are replicated. The noble genes with genome wide significant level ($p = 5x10^{-8}$) were 38 genes including C10orf2, CHRD, and CROCC. In the gene-based level using burden test, we selected genes with $p < 1x10^{-6}$. The well-known AD genes-ABCA7, SORL1, and TREM2-are replicated. The significant noble genes were 69 genes including AGAP1, ALYREF, and TYRO3. We not only replicate known AD risk genes-ABCA7, MS4A6A, SORL1, and TREM2, but also found the noble candidate AD risk genes. Our augmentation methods can be applied to the whole exome sequencing studies on other diseases.

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P09.005A

Assessing and challenging the somatic variant hypothesis in sporadic Alzheimer disease

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Introduction: In sporadic Alzheimer disease (sAD), highly penetrant pathogenic variants have been reported in the autosomal-dominant genes *APP*, *PSEN1* and *PSEN2* in a minority of early-onset cases (onset before 66 years), some of them occurring as *de novo* germline events. Given the recent knowledge on seeding and spreading of neuropathological lesions in AD, we hypothesized that somatic variants in these genes may contribute to sAD etiology in a proportion of patients with no germline pathogenic variant.

Methods: We applied an ultra-sensitive technique of single-molecule molecular inversion probes (smMIP)-based deep NGS to 100 brain and 355 blood samples from 445 sAD patients from France, the Netherlands and the UK, including 83.5% early-onset cases. The panel included *APP*, *PSEN1*, *PSEN2*, genes with a *de novo* germline mutation in a trio study (*VPS35, MARK4*), the risk factor gene *SORL1* and 5 genes involved in APP processing.

Results: We identified 9 somatic variants in 2 brain and 7 blood samples, with variant allele fractions ranging from 0.2% to 10.8%; 6/9 had a ratio below 0.5% (0.22-0.48%). All nine were confirmed by independent amplicon-based deep sequencing with similar fractions. Two somatic variants mapped to *APP*, although they were interpreted as likely benign. The other somatic variants were located in *SORL1* (n = 5), *NCSTN* (n = 1) and *MARK4* (n = 1). Two of the *SORL1* variants might have contributed to the disease while the other variants remain of unknown significance.

Conclusion: Somatic variants in the autosomal dominant AD genes may not be a common cause of sAD, including early-onset cases.

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Leukocyte telomere shortening is associated with progressive cognitive decline in amnestic mild cognitive impairment and Alzheimer's disease

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Introduction: Numerous studies have reported an association between shortened leukocyte telomere length (LTL) and increased risk of Alzheimer's disease (AD). In this study we investigated the relationship between LTL and AD development, including in the analysis patients with amnestic mild cognitive impairment (aMCI), a clinical entity considered prodromal of AD.

Materials and Methods: LTL (T/S ratio) was measured in patients with AD ($n = 61$) or aMCI ($n = 46$), and compared with LTL of age-matched controls ($n = 56$).

Results: significant LTL differences were observed between controls, aMCI and AD patients ($p < 0.0001$), with mean LTL values ($\pm$ s.d) in the order: AD patients (0.70 ± 0.15) < aMCI patients (0.80 ± 0.14) < controls (0.88 ± 0.15). A positive relationship (linear regression $p = 0.004$) was observed between LTL and cognitive performance (measured by Mini Mental State Examination score). LTL did not differ by apolipoprotein E (APOE) genotype.

Conclusions: The shortened LTL observed in AD patients appears to stem from progressive telomere erosion possibly correlated with the cognitive decline characterizing conversion from aMCI to AD. LTL reduction, indicating active cell proliferation, may reflect immune system involvement in AD pathogenesis.

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Contribution to Alzheimer's disease risk of rare variants in *TREM2*, *SORL1* and *ABCA7* in 1,779 cases and 1,273 controls

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Introduction: Alzheimer disease (AD) is a complex disorder with high genetic component. Associations of rare variants with AD risk have recently been reported, however the limited panels of variants under scrutiny or the sample sizes restricted the possibility to assess their effect fully.

Materials and Methods: We took advantage from the ADES-FR dataset to focus on *TREM2*, *SORL1*, *ABCA7* genes. We generated whole-genome (n-955) or whole-exome ($n = 2,106$) sequencing data and performed gene-based burden association analyses on 927 late-onset AD (LOAD, onset > 65 years) cases, 852 early-onset (EOAD, onset ≤ 65 years) cases and 1,273 controls.

Results: When aggregating rare (MAF<1%) protein truncating (PT) and missense variants predicted damaging by three bioinformatics tools (strictly damaging, SD), association with EOAD risk reached exome-wide significance ($p < 2.5x10^{-6}$). Missense variants predicted damaging by less than three bioinformatics tools were not associated with EOAD risk. No exome-wide significant signal was detected in the LOAD sample.

Conclusion: Beyond a confirmation of the association of *TREM2*, *SORL1* and ABCA7 rare variants with AD risk, our study provides a clearer insight into the classes of rare variants involded, namely SD variants sharing a common loss of function mechanism with PT variants, and sheds light on the genetic heterogeneity of AD. Despite different effect sizes and varying cumulative MAF, *TREM2*, *SORL1* and *ABCA7* rare PT and SD variants contribute similarly to the heritability of EOAD and explain between 1.1 and 1.5% of EOAD heritability each, compared with 9.12% for APOEε4.

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miR-146a and miR-181a are potential biomarkers for the progression of mild cognitive impairment to Alzheimer's disease

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Mild cognitive impairment (MCI) is a transitional stage between normal aging and Alzheimer's disease (AD). Not all MCI subjects convert to AD but some remain MCI, and the identification of biomarkers that could give alarm for dementia development would be of great usefulness in the clinical practice. MicroRNAs (miRNAs) are small non-coding RNAs that play a pivotal role in gene expression and in many neuronal mechanisms going to synaptic plasticity, neurogenesis, neurodegeneration and apoptosis. In this study we investigated if baseline blood levels of a set of candidate miRNAs (miR-22, miR-24-3p, miR-101, miR-146a, miR-181a, miR-181b, miR-186, miR-339, and miR-590) may be associated to AD conversion after 2 years in a group of 45 MCI patients (of whom 19 were converted). Expression level of miR-146a ($p = 0.$ 0.036) and miR-181a ($p = 0.$ 0.026) showed a significant upregulation in MCI subjects that converted to AD. A significant negative correlation was found between levels of miR-146a ($p = 0.006$) and miR-181a ($p = 0.001$) in blood and Aβ-42 concentration in CSF, while no association was evidenced for P-Tau, and T-Tau. Moreover the increase in miR-146a was associated with volume reduction in hippocampus and its subfields (CA1 $p = 0.013$, subiculum $p =$ 0.027) and increased levels of miR-146a ($p =$ 0.031) and miR-181a ($p =$ 0.002) were correlated with diffusivity alterations in the cingulum, a white matter tract connecting temporal with frontal and parietal lobes. In conclusion, the data obtained support a possible usefulness of blood miR-146a and miR-181a levels as biomarkers for illness progression in MCI patients.

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Genomic variants in the *FTO* gene are associated with sporadic amyotrophic lateral sclerosis in Greek patients

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Amyotrophic lateral sclerosis (ALS) is a devastating disease whose complex pathology has been associated with a strong genetic component in the context of both familial and sporadic disease. Herein, we adopted a stepwise approach, including whole genome and conventional Sanger sequencing, in order to explore further the genetic basis of sporadic ALS (sALS) in 3 patient cohorts of Greek (n = 150), Turkish (n = 148) and Sardinian (n = 114) origin. Whole genome sequencing yielded a total of 174 variants that were found in 6 Greek sALS patients and in none of the 5 ethnically-matched controls, mostly intergenic (n = 144) and intronic (n = 23) variants. Next, genes were clustered by metabolic or disease network and the most prominent ones were further analyzed in the entire 3 patient cohorts. Our analysis revealed a positive association between *FTO* gene variants and sALS in the Greek patient cohort, while linkage disequilibrium analyses were suggestive of a specific disease-associated haplotype for *FTO* gene variants. In addition, qRT-PCR analysis of fibroblasts, undifferentiated embryonic stem cells (ESCs), astrocytes, neural PAX6+ progenitors, as well as stem cell-derived spinal cord motor neurons (MNs) and cortical neurons (CNs) indicated that *FTO* gene expression is relatively neuron-specific and notably, is most highly expressed in motor neurons. To our knowledge, this is the first study to present a possible association between *FTO* gene variants and the genetic etiology of sALS, while the lack of association between *FTO* variants and sALS in patients of Sardinian and Turkish descent may suggest a founder effect in the Greek population. <!--EndFragment-->

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Polymicrogyria in patient with Angelman Syndrome-coincidence or a new feature ?

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Angelman syndrome (AS) is a neurogenetic imprinting disorder attributable to the reduced expression of maternally inherited *UBE3A* gene on chromosome 15. Angelman syndrome is characterized by a combination of severe intelactual impairement with limited speech, epilepsy, ataxic gait, unique behavior and psychiatric comorbidities. MRI usually reveals only a small sized central nervous system or minor abnormalities, such as mild cortical atrophy, dysmyelination and focal white matter signal abnormalities. To our knowledge there is no patient with polymicorgyria in previously reported patients with Angelman syndrome. Polymicrogyria is genetically heterogeneous, and only in a small minority of patients, a definite genetic cause has been identified. Here we report the patient with Angelman syndrome and polymicrogyria. The patient was born at term, birth measurements were normal. At the neonatal period he was presented with hypotonia. Brain MRI reavealed polymicrogyria. At the age of eight months, because of developmental delay and slight dysmorphic features he was referred to geneticist. Whole-genome oligonucleotide microarray analysis revealed a 5.75 Mb deletion of chromosome 15q11.2q13.1 (15:22765628-28520313; GRCh37) encompassing the critical region for AS/PWS. Further MS-MLPA test identified hypomethylation of SNRPN and MAGEL2 locus as well as confirmed the *maternal Class* I *deletion* extending from *BP1 to BP3*. FISH studies in both parents gave normal results, proving the de novo occurrence of this aberration in the child. Further studies (NGS) are needed to determine the possible

genetic causes of polymicrogyria in our patient.aCHG and MLPA studies were performed in Department of Medical Genetics, Chidren' s Memorial Health Institute.

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Two canine anxiety phenotypes overlap human neuropsychiatric loci

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Introduction: Anxiety disorders include a large spectrum of heterogeneous conditions and rank among the most common health concerns in human medicine. Anxiety disorders are known to be heritable, but genetically complex. Dogs suffer from various naturally occurring breed-specific compulsions and phobias such as noise sensitivity and fear, which respond to human anxiolytics and can be measured. This study aimed to find new anxiety loci in dogs.

Materials and Methods: A total of 330 German Shepherd dogs were phenotyped for two anxiety traits, noise sensitivity (NS) and fear towards novel humans and situations (fear) using a behavioral survey. Each dog was given a score describing the severity of the phenotype, ranging from 0 for controls to 1-60 for NS and 0.5-13.5 for fear. The dogs were genotyped using Illumina's canine HD SNP arrays and analysed for phenotype-genotype associations using single-locus method (PLINK) and single-locus mixed model approach (GenABEL).

Results: Genomic regions on chromosome 20 and chromosome 7 were significantly associated with NS and fear, respectively. The NS locus includes known anxiety and hearing related genes, such as the glutamate receptor 7 gene. The fear locus was syntenic to a locus in human 18p11 that has been linked to psychiatric illnesses.

Conclusions: The findings revealed new anxiety loci in dogs overlapping several genes associated with human neuropsychiatric disorders. Further investigation of the causative variants within the loci has a potential to shed light on the biological basis of the disorders in both species.

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A novel variant in *CACNA1G* is associated with early onset cerebellar ataxia

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Hereditary cerebellar ataxias (CAs) are a genetically heterogeneous group of disorders with different inheritance patterns and a variety of neurological symptoms. To date, more than 40 loci have been identified for the autosomal dominant forms. Here, we describe a 10 year-old girl who, at the age of 2 years, presented with mild motor delay, gait instability, dysarthria and oculomotor symptoms. Brain MRI showed mild cerebellar atrophy. The family history is negative. We performed targeted exome analysis and identified a heterozygous *de novo* missense variant in the *CACNA1G* gene which has not been described previously and is classified as likely pathogenic, according to the actual ACMG-guidelines. CACNA1G encodes Cav3.1, a T-type calcium channel belonging to the family of voltage-gated calcium channels. Cav3.1 is highly expressed in cerebellar neurons as well as in thalamic relay neurons. The variant described here (c.5152C>G; p.(Arg1718Gly)) induces an amino acid change in the voltage sensor S4 segment of Cav3.1. It is located in close proximity to the recurrent *CACNA1G* missense variant c.5144G>A, reported in French and Japanese families with CA (Coutelier et al. and Morino et al., both 2015). The previously reported patients with the recurrent *CACNA1G* variant all presented with symptom onset in adolescence or adulthood. The present case highlights that *CACNA1G* has also to be considered in children with CA with very early onset. In analogy to episodic ataxia type 2, which is caused by variants in *CACNA1A*, we started a treatment with acetazolamide and will report on the efficacy thereof.

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An unstable ATTTC repeat mutation within the Disabled 1 gene causes cerebellar Purkinje cell alterations, DAB1 RNA

switch, and Reelin-DAB1 signalling dysregulation in Spinocerebellar ataxia type 37

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The spinocerebellar ataxias (SCAs) are characterised by progressive cerebellar ataxia variably associated with ophthalmoplegia, pyramidal and extrapyramidal signs, dementia, pigmentary retinopathy, seizures, lower motor neuron signs, or peripheral neuropathy. We previously reported a novel spinocerebellar ataxia subtype, SCA37, linked to an 11-Mb genomic region on 1p32 in a large Spanish ataxia pedigree, characterised by a pure cerebellar syndrome distinctively presenting with early-altered vertical eye movements. Here we demonstrate the segregation of an unstable intronic ATTTC pentanucleotide repeat mutation within the 5'-noncoding regulatory region of the gene encoding the reelin adaptor protein implicated in neuronal migration DAB1, as the causative genetic defect of the disease in four Spanish SCA37 families. Neuropathology revealed severe loss of Purkinje cells (PCs) with abundant astrogliosis, empty baskets, occasional axonal spheroids, and hypertrophic fibers by phosphorylated neurofilament immunostaining in the cerebellar cortex. The remaining PCs showed loss of calbindin immunoreactivity, aberrant dendrite arborisation, nuclear pathology, and multiple ubiquitinated perisomatic granules immunostained for DAB1. A subpopulation of Purkinje cells was found ectopically mispositioned within the cerebellar cortex. Importantly, we demonstrate that the ATTTC repeat mutation dysregulated DAB1 expression and induced a DAB1 RNA switch resulting in the up-regulation of Reelin-DAB1 and PI3K/AKT signalling in the SCA37 cerebellum. This study reveals the unstable ATTTC pentanucleotide repeat mutation within the *DAB1* gene as the underlying genetic cause and provides evidence of cerebellar Reelin-DAB1 signalling dysregulation in the spinocerebellar ataxia type 37. This work was funded by the Spanish Health Institute Carlos III (CP14/00029; FIS PI14/00136; PI14/01159; FIS PI17/00534).

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A novel intronic ATM gene mutation affecting splicing in a patient with Ataxia-Telangiectasia

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Introduction: Ataxia-telangiectasia (AT) is a rare autosomal recessive disorder characterized by progressive ataxia, chorea, myoclonus beginning in early childhood. The other characteristic feature of the disease is telangiectases in the eyes and also on the skin. *ATM* gene mutations which encodes a protein involved in cell division and DNA repair, are resposible for AT.

Method: After the clinical evalution of the case next generation sequencing was performed for *ATM* gene analysis. Detected intronic mutation was investigated for splicing affect. From peripheral blood lymphocytes mRNA was isolated and via revers transcription cDNA was obtained. *ATM* gene exon 48-50 was sequenced using spesific primers targeting related region.

Result: A 6 year-old girl was referred us with the diagnosis of AT. She was born at term to consanguineous parents. She had gait disturbance and dysartria for 3 years. Multiple cutaneous telangiectases were observed on her face, trunk and limbs. Next-generation sequencing analysis of *ATM* gene revealed homozygous c.7308-15A>G variation in IVS49. Parents were carrying the variation in heterozygous site. Human Splicing Finder predicted that the mutation could activate an intronic cryptic acceptor site. We designed primers for amplification of related exons (49-50) from cDNA for evaluating splicing pattern. A fourteen nucleotide insertion from intron 49 were detected between exon 49 and 50, resulting premature termination of translation at codon 2439.

Conclusion: In this study we investigated the affect of an intronic mutation on splicing and revealed the molecular diagnosis of the AT case. <!--EndFragment-->

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Appropriateness of genetic testing in the ADHD clinics: a comparative study

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Introduction and objective: Attention Deficit Hyperactivity Disorder (ADHD) is a common and heritable neurodevelopmental disorder characterized by persistent inattention, hyperactivity and impulsivity. ADHD is frequently comorbid with other neuropsychiatric disorders. Array-CGH is the first-tier genetic test for patients with idiopathic autism and intellectual disability with a reported diagnostic yield ranging from 4% to 30%. Yet, its utility in the ADHD clinics is more controversial. The aim of this study was to compare the array-CGH diagnostic yield in 91 ADHD subjects divided into two groups according to the clinical diagnosis: (1) 48 subjects diagnosed with ADHD as primary diagnosis, co-morbid with learning disabilities, conduct disorders, motor coordination disorders, oppositional defiant disorders and mood disorders (2) 43 subjects in which ADHD was co-morbid with autism and/or intellectual disability.

Materials and Methods: Array-CGH technology was performed using the Human Genome CGH SurePrint G3 Microarray 4x180K Kit (Agilent).

Results: We detected pathogenic and likely pathogenic CNVs in 37% (16/43) subjects in which ADHD was co-morbid with autism and/or intellectual disability and in 21% (10/48) subjects diagnosed with ADHD as primary diagnosis (exact P=0.105, n.s.). Detection of CNVs of unknown clinical significance was similar in the two groups being 10% and 8% in group (2) and in group (1) respectively.

Conclusions: Array CGH is a valuable diagnostic tool to detect pathogenic and likely pathogenic CNVs in ADHD-affected subjects, even in the absence of comorbidity with autism and/or intellectual disability, although the latter may be associated with significantly greater diagnostic yield with a larger sample size.

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Determination of prospective genes for autism spectrum disorders using microarray

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Introduction: Copy number variants (CNVs) play an important role in susceptibility to autism. Clinical significance, however, is still unclear in many of them. Gene content of CNVs seems to be crucial for determination of the significance.

Aim of the study: Identification of prospective candidate genes that could play a role in the aetiology of autism by microarray.

Patients and Methods: CNV analysis (Cytoscan HD Affymetrix, CytoSNP-12 Illumina) was performed in 93 patients of Caucasian ethnicity - 63 males and 30 females - with autism, PDD-NOS and Asperger's syndrome, predominantly from simplex families. Systematic analysis of the genes involved in CNVs was performed using databases Decipher, OMIM, DGV and SFARI databases.

Results: We detected 188 OMIM genes affected by CNV. 28 of them are associated with neurodevelopment disorders in OMIM database. Genes ARX (SFARI score S), EIF2S3, GAL, GNAS, GRN, KLHL15, MSX2, NRXN1 (SFARI score 2), ORC6, PRODH, PTCHD1 (SFARI score 2), POLA1, SOX3, TSPAN7 and ZIC1 are associated with autosomal dominant diseases with mental retardation, intellectual disability, schizophrenia, agenesis of the corpus callosum, frontotemporal lobar degeneration, craniosynostosis, and lissencephaly, epilepsy and seizure.

Conclusions: In our cohort we identified several new possible candidate genes associated with autism (EIF2S3, GAL, GRN, KLHL15, MSX2, ORC6, PRODH, POLA1, SOX3, ZIC1), in addition to previously reported ones (ARX, GNAS, NRXN1, PTCHD1, TSPAN7). However, further study is essential for determination of their significance in aetiology of ASD.

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An unusual high frequency of natural fetal loss in a Colombian cohort with Autism Spectrum Disorder

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Autism spectrum disorders (ASD) are neurodevelopmental disorders that share difficulties in communication, social interactions and stereotyped behaviors. ASD has a heritability of 64 - 91% indicating a high genetic component. Clinically recognized pregnancy loss is relatively common in the population with an estimate between 12-20%. However, this risk increases substantially, frequencies between 58-65%, for genetic diseases such as Edwards and Patau syndromes. Here we describe an unusual high rate of previous natural fetal losses in a cohort clinically diagnosed with ASD. We have clinically ascertained 45 family trios composed of mother, father and child with autism, of which 44% (20 mothers) had previous natural fetal losses; 14 of them had one previous natural fetal loss, and 6 mothers had two or more previous natural fetal losses. Age was not a critical factor in our cohort suggesting that clinically recognized pregnancy loss might be associated with increases risk of autism.

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Significance of submicroscopic chromosomal copy-number variants in etiopathogenesis of autism spectrum disorders

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Autism Spectrum Disorders (ASDs) are one of the most prevalent groups of neurodevelopmental disorder that affects around 1-2% of the population with the average male to female ratio 4-5:1. A strong genetic contribution to the etiology of ASDs has been recognized in ~ 25-40% of patients. In ~ 7-14% of individuals with ASDs, submicroscopic chromosomal copy-number variants (CNVs) are one such contributing factor. Because of the large genetic heterogeneity of ASDs, high-resolution whole-genome analyses such as aCGH are useful tools to study the etiopathogenesis of these disorders.

We applied genome-wide oligonucleotide microarrays (OGT) with average resolution 30 kpz for identification and characterization of CNVs in patients with ASDs. The analyses of the patients' genomes were performed using arrays contained approximately 180,000 oligonucleotide probes that covered the entire human genome and allow to accurate detection of copy number variation at the exon level. For the study 95 patients were qualified.

Chromosomal microarray analysis revealed 18 nonpolymorphic CNVs, ranging in size from 15 kb to 3.1 Mb, in 17 (17.9%) patients. We identified pathogenic or potentially pathogenic CNVs in 9 individuals with ASDs (9.5%), whereas CNVs with unknown clinical significance were identified in 9.5% of cases. All of the identified CNVs were submicroscopic in size and therefore could not have been detected by standard karyotype analysis. Our results confirmed the importance of array CGH in detection of CNVs in patients with ASDs.

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Facial dysmorphisms as biomarkers for autism spectrum disorder

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Introduction: Current diagnosis of autism spectrum disorder ASD is based on behavioral assessment that complicates diagnosis. Many genetic syndrome that share comorbidities with ASD, have unique facial dysmorphisms.

Therefore, we hypothesized that children with ASD have distinct facial characteristics that could facilitate diagnosis of the disorder.

Methods: Cases included children with ASD from the Negev HUB autism database. Frontal facial photos of these children were compared to those of normally developed children (matched by age, sex and ethnicity at a 2:1 ratio). A deep convolutional neural network (DCNN) architecture with batch normalization was used to evaluate these photos. Classification accuracy between the groups was assessed using cross-validation approach with 90% of the photos using for training, and 10% for validation. Permutation analysis with 1000 replication was used to assess the statistical significance of group classification.

Results: Overall, 82 children with ASD (78% males, and 28% Bedouin) with a mean age of 4.78 ± 2.03 years participated in the study. The FDNA algorithm could distinguish between ASD and controls with a 96.6% accuracy (AUC=0.966; 95%CI=0.964-0.968(. This was remarkably better than the classification accuracy of gender or ethnicity (AUC=0.706 and AUC=0.790 respectively). Analysis of upper face achieved better separation between cases and controls than lower face (AUC=0.892 vs. AUC=0.728), with eyes and nose being the most distinct facial characteristics (AUC=0.912 and AUC=0.919 respectively).

Conclusions: Our findings suggest that children with ASD have unique facial characteristics that could be used as biomarkers for the disorder.

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A role for gene-environment interactions in Autism Spectrum Disorder is suggested by an excess of potentially pathogenic variants in genes regulating exposure to toxicants

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Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder with multifactorial etiology. Genetic factors are strongly implicated in ASD, while environmental toxicant exposure early in development is a documented risk factor, suggesting a role for gene-environment interactions. We thus examined whether CNVs and SNVs in genes involved in regulation of toxicant exposure, namely in detoxification processes and physiological permeability barriers, occur more frequently in individuals with ASD than in control subjects. For this purpose, publicly available genomic datasets (AGP, SSC, ARRA, DGV) and the Comparative Toxicogenomics Database were analyzed. CNVs in 8 genes (*STS*, *CYP2D6*, *ARSF*, *GUSB*, *CLDN3*, *CYP2R1*, *SLC3A2* and *SULT2B1*) were found exclusively in ASD subjects, while CNVs in 7 genes (*CSH1*, *MAGEA8*, *CYP4X1*, *CHST5*, *CSH2*, *GH2* and *ABCC1*) were more frequent in ASD individuals than in controls, after correction for multiple testing ($6.04 \times 10^{-13} < P < 1.37 \times 10^{-5}$). All these genes also carried detrimental loss-of-function or missense variants. Rare *de novo* loss-of-function or missense SNVs were further identified in the *AR* and *ANKRD11* ASD candidate genes, and also in the *CBS*, *CES1, GUSB* and *JUP* genes. Most of these genes interact with toxicants implicated in ASD, namely bisphenol A, heavy metals and benzo(α)pyrene, and are key players in detoxification processes or regulation of blood-brain barrier and placenta permeability, which are crucial in controlling exposure during development. Notably, the hormonal regulation functions of molecules encoded by *STS*, *AR*, *CSH1*, *CSH2* may link toxicant-related endocrine disruptions with the high ASD male/female ratio. These findings thus reinforce the hypothesis that gene-environment interactions contribute to ASD.

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miRNA and lncRNA gene variants in Autism Spectrum Disorder

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Autism Spectrum Disorder (ASD) is a clinically heterogeneous neurodevelopmental disorder. Genetic factors are estimated to account for 50 to 80% of the familial ASD risk, but most of the genetic determinants are still not known and a role for epigenetic factors is likely.

In this study we explored the potential role of noncoding RNAs in ASD by comparing the frequency of Copy Number Variants (CNVs) targeting microRNA (miRNA) or long noncoding (lncRNA) genes in ASD patients (n = 3570) with control subjects (n = 9649), using the Fisher's exact test corrected for multiple testing.

We found 22 miRNA genes exclusively targeted by CNVs in ASD subjects and 14 miRNA genes more frequently disrupted by CNVs in ASD patients than in controls. Two miRNA were previously associated with ASD in serum miRNA profiling studies, while 5 novel miRNAs for ASD have been described in schizophrenia, a disorder that phenotypically and genetically overlaps with ASD. Many putative targets of these 36 miRNAs are reported ASD risk genes. Gene-target enrichment analysis identified 6 significant pathways, 2 of which, the PI3K-Akt and MAPK signalling pathways, have been implicated in ASD. We further identified 102 novel lncRNA genes more frequently targeted by CNVs in ASD, 3 of which are antisense to ASD candidate genes.

These results support our hypothesis that genetic variants targeting noncoding regulatory RNAs are involved in ASD pathophysiology. This systems biology integrative strategy will provide a better understanding of the biological processes underlying ASD, and contribute to biomarker and drug target discovery.

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Copy number variation analysis in autism spectrum disorders

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Introduction: Although the proportion of heredity in autism spectrum disorders (ASD) is estimated to be as high as 90%, genetic factors can only be detected in 20-25% of cases because of their heterogeneity and complexity. Among these factors, CNVs come to the forefront with a high level of 10% and being easily detectable.

Methods: Array-CGH analysis (Agilent ISCA 8x60K) was performed in 30 patients with non-syndromic ASD.

Results: Pathogenic CNVs (P) were found in 4 (13%) patients, clinically uncertain CNVs (VUS) in 2 (6,7%) and VUS/likely pathogenic CNVs (VUS-LP) in 3 (10%; Table).

Conclusions: The presence of P/ VUS-LPs in 23% of patients indicated the importance of CNVs in ASD etiology and that microarray should be used as the first step in the diagnostic algorithm. Deletion of 22q13.3 detected in two patients (6.7%) are common pathogenic anomalies in ASD. The LRCC7, NRXN1 (intronic region) and MACROD2 genes identified in VUS-LP regions have previously been shown to be related with autism. So, we suppose that VUS-LPs detected in this study may be a direct cause or low penetrating risk factor for ASD.

Table: CNVs Identified With Array-CGH in Patients with ASD

Patient No	Cytoband	Start-Stop (bp)	Size (kb)	Genes	Del / Dup	Inheritance	Interpretation
Pt2	22q13.33	50241153-51178264	930	ALG12, MLC1 SBF1, SCO2, ARSA, SHANK3, ACR...	Del	De novo	Pathogenic
Pt5	1p31.1	70059561-70386514	326	LRRC7, PIN1P1	Del	Unknown	VUS/ Likely pathogenic
Pt10	2q36.3	230479578-230951771	472	DNER, TRIP12, FBXO36, SLC16A14	Dup	Paternal	VUS
Pt13	9p24.3 p24.2	204193-3221675	3,017	DOCK8, KANK1, DMRT1, DMRT2, SMARCA2, VLDLR…	Del	Maternal (mildly affected)	Pathogenic
Pt19	22q13.33	51123491-51178264	55	SHANK3, ACR	Del	De novo	Pathogenic
Pt22	4q25q26	113762831-114217645	455	ANK2, MIR1243	Del	Unknown	VUS
Pt23	2p16.3	50937444-51054432	117	NRXN1 (intronic)	Del	Maternal	VUS/ Likely pathogenic
Pt27	20p12.1	14567155-14700099	133	MACROD2	Del	Unknown	VUS/ Likely pathogenic
Pt28	1q21.1 q21.2	146507518-148545520	2,038	FMO5, CHD1L, BCL9, GJA5, GJA8, GPR89B…	Del	De novo	Pathogenic

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P09.024D

Accessing mRNA and miRNA expression in binge eating disorder

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Binge Eating Disorder (BED) is a disorder commonly associated with obesity, diabetes, cardiovascular diseases and psychiatric disorders such as depression and anxiety. Although the disorder presents a genetic influence, genes involved in risk for BED remain unknown. The aim of the current project was to analyze mRNA expression of neuroplasticity and neurotransmission genes (*SLC6A4* and *BDNF)* and miRNAs *(miR16* and *miR206)* possibly related to their regulation in BED patients and healthy controls. Fifty five patients with BED and twenty eight control subjects, matched for age and sex, were analyzed. Peripheral blood of all individuals was collected and total RNA was extracted from white blood cells using commercial tools. The assessments of mRNA and miRNAs were performed using qRT-PCR technique with TaqMan detection method. We did not detect *miR206* expression in the blood samples of both groups. In the comparison between BED and control group, BMI differences were detected, with increased values of BMI being observed in BED subjects. We observed differences in *BDNF* gene expression between BED and healthy controls, however, these results did not remain different after correction for BMI values. No differences in *SLC6A4* and *miR16* expression were observed between groups. Although these target genes were pointed as candidates involved in BED patophysiology, the absence of differences in gene expression between groups does not indicate the use of these genes as BED biomarkers.

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Analysis of the influence of microRNAs in Lithium Response in Bipolar Disorder

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Bipolar disorder (BD) is a common, highly heritable neuropsychiatric disease characterized by recurrent episodes of mania and depression. Lithium represents the best-established long-term treatment for BD, even though individual response is highly variable. The largest GWAS of lithium response to date conducted by the International Consortium on Lithium Genetics (ConLiGen) provides evidence for the genetic basis of this variability. The first genome-wide analysis of involvement of miRNAs in BD identified nine BD-associated miRNAs, however it is unknown whether these miRNAs are also associated with lithium response in BD. We therefore tested whether common variants at these nine candidate miRNAs contribute to the variance in lithium response. Furthermore, we systematically analyzed whether any other miRNA is implicated in the response to lithium. We performed gene-based tests for all known miRNAs in the ConLiGen GWAS dataset (n = 2,563 patients) using a set-based testing approach adapted from VEGAS2. In the candidate approach, *miR-499a* showed nominally significant association with lithium response, thus providing evidence for involvement in both development and treatment of BD. In the genome-wide miRNA analysis, 71 miRNAs showed nominally significant associations with the dichotomous and 106 with the continuous trait for treatment response. 15 miRNAs revealed nominal significance in both phenotypes with *miR-633* showing the strongest association with the continuous ($p = 9.80E-04$) and *miR-607* with the dichotomous phenotype ($p = 5.79E-04$). No association between miRNAs and treatment response to lithium withstood multiple testing correction. Given the limited power of our study, the investigation of miRNAs in larger samples of BD and lithium response is warranted.

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P09.026B

Rare genetic variants in ion channels genes identified by NGS contribute to both schizophrenia and bipolar disorder

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Background: Genes associated with ion channels were shown to contribute to schizophrenia (SCZ) and bipolar affective disorder (BAD). Targeted NGS of a panel of genes, including ion channel genes, was performed to investigate the genetic architecture of Bulgarian patients with BAD and SCZ.

Methods: A total of 300 individuals with BAD, 151 with SCZ, 15 SAD, diagnosed with DSMIV and ICD-10, 85 controls and 40 healthy relatives were recruited. Sequencing was done on Ion PROTON platform. The panel comprised of 187 candidate genes, 39 of them coding ion channel proteins. Only samples with coverage >95% of the target region at 20x were included in the analyses. Case-control association testing was done using PLINK. Prediction tools (SIFT&PolyPhen2) were used.

Results: The case-control analysis revealed no significant association with SCZ, SAD and BAD, that survived the correction for multiple testing. However, 681 rare variants present in affected only were found. 260 of them were recurrent and 421 were singletons. Of these, 4LoF, 218 missense, 63 of which potentially damaging, 30 splice and 7 regulatory variants were detected. The genes with most detected variants were *CACNA1H,CACNA1B* and *CACNA2D4*.

Discussion: No significant association of common variants with the diseases was found, probably due to limited power. However, recurrent and unique rare variants with potential functional relevance were detected, that deserve further investigation. This adds to the accumulating evidence that ion channelopathies may be involved in the pathogenesis of SCZ and BAD. The work was supported by projects DUNK01-2/2009 and B02/6/2014 funded by NSF, MYES

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Exome sequencing of multiplex bipolar disorder families and follow-up resequencing implicate rare variants in neuronal genes contributing to disease etiology

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Bipolar disorder (BD) is a highly heritable mood disorder with a lifetime prevalence of around 1%. Models of illness are most consistent with a polygenic contribution of common and rare variants to disease susceptibility. As the cumulative impact of common alleles may only explain around 25-38% of the phenotypic variance, rare variants of high penetrance have been suggested to contribute to BD susceptibility.

In this study, we performed whole-exome sequencing in 226 individuals of 68 large multiplex BD families of European origin. We filtered for rare (minor allele frequency < 0.1%), non-synonymous, potentially functional and segregating variants.

We identified 1214 variants implicating 1122 different genes. Gene enrichment analysis of 294 genes that were among the 20% most "intolerant" genes showed a significant enrichment for 18 pathways ($p < 0.001$) including neuron projection, axon development and cell-adhesion.

For follow up analyses, we prioritized genes that were found in at least two unrelated families in the present study, previously reported in next generation sequencing or GWAS studies of BD, or predominantly driving the significant pathways in our gene enrichment analysis. The different approaches of prioritization yielded 42 promising candidate genes including *SYNE1* which is a genome-wide significant BD risk gene.

The 42 prioritized candidate genes are currently being followed up by resequencing in larger cohorts of 2000 independent BD patients and 2000 controls of European ancestry using the single molecule molecular inversion probes technology.

Our preliminary results suggest that rare and highly penetrant variants in neuronal and cell-adhesion genes contribute to BD etiology.

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Bisphenol A Triggers ER stress Mediated Autophagy in SH-SY5Y cell line

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Introduction: Bisphenol A (BPA) is a chemical produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins. While BPA is found in a number of consumer products such as hard plastic drinking containers and the linings of infant formula and food cans, human are regularly exposed to this chemical. The potential cytotoxic and genotoxic effects of BPA have been thoroughly studied on neuroblastoma cells (SH-SY5Y).

Materials And Methods: SH-SY5Y cells were treated with 50 μM and 100 μM concentrations of Bisphenol for 48 hours. After RNA isolation and cDNA synthesis, Real-time PCR reactions were performed for GRP78, XBP1, BECN1 and ATG5 genes.

Results: In the present study, we aimed to investigate the potential effect of Bisphenol on unfolded protein response induced by Endoplasmic Reticulum Stress and Endoplasmic Reticulum Stress-Mediated Autophagy in neuron like cell line (SH-SY5Y). In accordance with this purpose we exposed to SH-SY5Y cell line with both different doses of bisphenol. After 48h exposure, we measured mRNA levels of the genes that play a role unfolded protein response and autophagy.

Conclusion: We observed a significant decrease in GRP78 (Glucose-Regulated Protein) mRNA level and a significant increase in XBP1(X-Box Binding Protein 1), BECN1(Beclin 1), ATG5 (Autophagy Related 5) mRNA level at low dose (50μM) Bisphenol exposure. According with this result we can conclude that 50 μM dose of Bisphenol induced the autophagy via ER stress in SH-SY5Ycell line.

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Cerebral MR imaging based genetic assessment of brain malformations

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Introduction: Structural brain malformations are an important cause of early psychomotor retardation, intellectual disability and seizures. Identification of the underlying mutations allows more precise genetic counseling of the affected families and may also provide additional information directly relevant for further diagnostic workup or therapeutic decisions.

Methods: Initial evaluation of available cMR images (56 patients) or imaging data, clinical data and individual genetic testing by either Sanger sequencing/MLPA (14 patients) or multi-gene panel sequencing (204 patients) after Nextera Enrichment (Illumina) and bioinformatic

assessment of called variants with our in house pipeline including SeqNext (JSI medical systems) and evaluation for CNV using an in house JAVA-based skript.

Results and Discussion: For 18 patients with typical cMRI pattern a single gene analysis was employed allowing to identify the causal L1CAM (6), LIS1/PAFAH1B1 (7), DCX (2) or TUBA1A (2) mutations. Overall causal mutations (ACMG class 5 or 4) were identified for 76 patients (35%) including: hydrocephalus 10/37; Walker-Warburg syndrome 9/12; Periventricular nodular heterotopia 2/7; Lissencephaly/Double cortex 20/28; Polymicrogyria 6/30; (Ponto)cerebellar hypoplasia 10/19; Holoprosencephaly spectrum 12/51 and Microcephaly 7/37. NGS panel sequencing substantially increased the number of identified variants of unknown significance (ACMG class 3), which were observed in 27% of the overall patient cohort. Three holoprosencephaly patients with heterozygous mutation in one of the core genes were heterozygous for an additional variant, suggestive for digenic inheritance. Our data emphasize the importance of the individual clinical and imaging data for the individual testing strategy and for the final clinical classification of identified sequence variants.

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P09.030B

Epileptic encephalopathy due to BRAT1 pathogenic variants: report of eight new patients

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In 2012, Puffenberger et al., reported the first description of a lethal neonatal rigidity and multifocal seizures syndrome (OMIM# 614498) in 5 patients from the Amish community. This syndrome is caused by bi-allelic mutations in *BRAT1* and characterized by early-onset epilepsy, arrest of head growth, severe muscular hypertonia, frequent apnea and bradycardia, feeding difficulties and early death due to cardiopulmonary failure. Microcephaly is inconsistent at birth but progresses quickly over time accompanied by progressive cerebral atrophy. EEG may show a suppression-burst pattern. Beside this severe presentation, a milder form has been described in 2016 by Srivastava et al., in 5 patients. It is characterized by ataxia and cerebellar atrophy, prolonged survival, less severe microcephaly and inconstant epilepsy. Twenty-eight individuals have been reported so far with bi-allelic mutations in *BRAT1*. We report here 8 new patients: two Algerian brothers with a homozygous c.2068G>T (p.Glu690*) variant; three German siblings compound heterozygous for c.228insA(p.Leu77Thrfs*114) and c.638insA (p.Val214Glyfs*189); and three unrelated French female girls, compound heterozygous for the following combination of variants c.458A>C (p.Gln153Pro) and c.294dupA (p.Leu99fs), c.294dupA(p.Leu99fs) and c.2125_2128del(p.Phe709fs), and c.359C>A(p.Arg120His) and c.1313_1314delAG(p.Gln4348Argfs*51). All but one patient died during the first 14 months of life. The surviving patient, aged 7 years, has a milder form. Five variants are novel: 3 nonsense and 2 missense. Although, no clear genotype-phenotype correlation has emerged, the presence of two nonsense mutations seems to produce a more severe phenotype than bi-allelic missense mutations.

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Regressive autism spectrum disorder expands the phenotype of BSCL2-associated neurodegeneration

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Loss-of-function variants of *BSCL2*, encoding seipin, were reported in congenital generalized lipodystrophy type 2, whereas two closely localized gain-of-function variants are linked to two distinct neurological phenotypes: distal hereditary motor neuropathy type V and hereditary spastic paraplegia type 17. In 2013, six Spanish patients affected with progressive encephalopathy death during infancy, and homozygous or compound heterozygous for a rare *BSCL2* exon 7 skipping variant (c.985C>T), were reported. We report on a female patient with regressive autism spectrum disorder who developed atypical parkinsonism in adulthood. She walked at the age of 11 months and began to associate words at 18 months. Slight behavioral disorders appeared by 3 years of age, evolving to invasive rituals, loss of communication and language skills, and sleep disorders. At the age of 6, a clinical examination disclosed motor stereotypies, trichotillomania and lower limb hypertonia. At the age of 16, Bichat's fat pads, strabismus, bilateral worsening of dystonic hypertonia, and extrapyramidal and pyramidal features were noted. At the age of 23, falls, dysphagia and a marked frontal lobe syndrome appeared. She died of a pulmonary infection at 28 years of age. Brain MRI and CIT SPECT showed bilateral dopaminergic denervation of the caudate nucleus. Trio-based whole-exome sequencing showed two *BSCL2* transitions. In addition to the c.985C>T transition previously reported she was compound heterozygous for the c.1004A>C transition that was also predicted to favor exon 7 skipping. The present observation shows that *BSCL2* pathogenic variants can cause severe autistic regression in infancy and lethal atypical parkinsonism in adulthood.

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Comparison of phenotypic variability with *C9orf72* gene GGGGCC hexanucleotide repeat expansion in frontotemporal lobar degeneration spectrum

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FTLD describes a group of progressive brain disorders. The expansion of a noncoding GGGGCC hexanucleotide repeat in the *C9orf72* gene is a major cause of both familial FTLD and amyotrophic lateral sclerosis (ALS).

The study was aimed to determine the prevalence of *C9orf72* GGGGCC repeat expansion in the Turkish population with FTLD and to determine the effects on the phenotype. The G4C2 expansion in *C9orf72* gene were analysed in 100 FTLD cases without mutations of *MAPT, PGRN,CHMP2B,VCP,TARDBP,FUS* genes and a hundred age-matched healthy controls by repeat-primed (RP-PCR) and size-PCR techniques.

The pathogenic expansion (>30) was found in one of the familial cases(1/33) but none of sporadic cases. The allele length difference between the cases and controls was statistically significant ($p < 0.01$). An intermediate (20-30) repeats was detected in 4% of our cases. The dominancy of intermediate/pathogenic repeats was seen in the cases with psychotic symptoms.

This is the first study in our knowledge to evaluate the *C9orf72* GGGGCC repeat expansion for the Turkish FTLD spectrum. As a result of our study, it is thought that *C9orf72* repeat expansion is not common in Turkish FTLD cases, but intermediate repeat may be an increased risk factor for FTLD or may act as a modifying gene. In addition, we believe that the correlation of these intermediate/pathogenic repeats with psychotic symptoms is prognostically important. Our data should be supported by further studies in different ethnic groups of FTLD patients from Turkey.

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P09.033A

Major intra-familial phenotypic heterogeneity encompassing epileptic encephalopathy due to a *CACNA1A* missense variant

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The *CACNA1A* gene encodes a calcium-dependent voltage channel, localized in neuronal cells. Pathogenic variants in this gene are known to lead to a broad clinical spectrum including episodic ataxia type 2 (EA2), spinocerebellar ataxia type 6, familial hemiplegic migraine, and more recently epileptic encephalopathy. We report a large family revealing wide variability of neurological manifestations associated with a missense mutation. The index case had early-onset epileptic encephalopathy with progressive cerebellar atrophy, although his mother and his great-grandmother suffered from paroxystic episodic ataxia. His grandfather and great grand-aunt reported no symptoms, but two of her sons displayed progressive late-onset spinocerebellar ataxia. Two of her little daughters also suffered from epilepsy. All these relatives were carriers of a heterozygous missense variant in *CACNA1A*: c.835C>T, p.(Arg279Cys) (NM_023035.2). This *CACNA1A* variant has already been described associated with EA2 phenotype. Prediction softwares suggested a damaging effect on the protein, and this variant was absent from GnomAD. Mutations in *CACNA1A* may lead to several different phenotypes, including severe epileptic encephalopathy which was only recently described. Loss-of-function mutations in *CACNA1A* associated with large phenotypic heterogeneity have rarely been described. We report here the first missense mutation segregating in a large family and leading to major clinical variability with incomplete penetrance. Environmental factors and modifier genes may have influences on the phenotype. Our family highlights difficulties to provide accurate genetic counselling concerning prenatal diagnosis in regard to highly variable severity of the clinical spectrum and incomplete penetrance.

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Comprehensive genetic analyses implicate highly heterogeneous etiology of idiopathic cerebral palsy spectrum disorders

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Cerebral palsy (CP) is an umbrella concept spanning a range of symptoms albeit with the common feature, i.e., an inborn disturbance of human movement and posture. CP is observed with consistent prevalence regardless of the socioeconomic advancement, and familial CP cases were reported. Seminal studies have confirmed the involvement of genetic changes, in the forms of copy number variant (CNV), insertion and deletion (Indel), and single-nucleotide variant (SNV). As a major children's medical center in China, we recruited a CP cohort of >500 individuals with multiple subtypes and severity. To elucidate the genetic causality, we initiated a pilot study of 120 idiopathic CP patients with their parents and siblings, with a comprehensive genomic assessment. We considered possible etiologic mechanisms from chromosomes to genes via multiple high-throughput platforms. We found that up to 45% of CP cases could be due to detrimental mutations. Causative *de novo* mutations were more prevalent than inherited ones, and post-zygotic mutations (PZM) took a nonnegligible portion. Major factions of the causal mutations were SNVs and indels in nuclear genome, while mitochondrial genome harbored several putative mutations, and large segmental alterations accounted for 8% of CP cases. Strikingly, besides the conventional nonsynonymous and splicing-site mutations, we observed by whole-genome sequencing a host of mutations residing in the regulatory regions which might serve as culprit for disease. Our work is of critical significance to reveal the underlying reasons of CP, which is among the major causes for children referred to the neuropediatric departments and rehabilitation institutions.

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Replication stress-mediated chromosome instability in the Alzheimer's disease brain

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Introduction. Chromosome instability (CIN) was recently suggested as a mechanism for neurodegeneration. However, origins and underlying causes of CIN in the brain remain obscure. To gain further insights into origins of brain-specific CIN, we have addressed intercellular and inter-individual genome variations in the Alzheimer's disease (AD) brain.

Materials and Methods. Using FISH-based techniques (multiprobe/quantitative FISH and interphase chromosome-specific multicolor banding), we have analyzed 14 AD post-mortem brain samples. Additionally, we have evaluated CNV by array CGH and an original bioinformatic technique (Iourov et al., 2014; Yurov et al., 2017) for identifying possible CIN origins.

Results. CIN affected 5.2-20.3% of brain cells in the samples. CIN essentially manifested as aneuploidy, structural chromosome rearrangements affecting mainly chromosome 21, chromosomal breaks resulting in the presence of 1-4 acentric chromosomal fragments in a nucleus. Further genomic analyses have shown that a CNV burden enriched for cell cycle and similar pathways is observed in the AD brain. Moreover, the distribution of genes affected by CNV has indicated that there is a significant bias toward genes involved in the DNA replication pathway.

Conclusions. Previously, we proposed the DNA replication stress hypothesis of AD (Yurov et al., 2011). Here, an empirical support of this hypothesis seems to be provided. Nonetheless, further studies are required to highlight replication stress as a mechanism and a therapeutic target for neurodegeneration treatment. Supported by ERA.Net RUS Plus Programme.

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Evaluation of CNVs variants in a cohort of isolated and syndromic intellectual disability/autism spectrum disorders reveals novel position effects and candidate disease genes

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Array-comparative genomic hybridization (array-CGH) is widely used to detect copy number variants (CNVs) associated with intellectual disability (ID) and autism spectrum disorders (ASDs).

However, some CNVs have no clear causative effects and remain of uncertain significance (VOUS).

We retrospectively reviewed the results of diagnostic array-CGH test on 700 cases with isolated or syndromic forms of ID and ASDs. We assessed pathogenicity of identified CNVs by combining mining of literature and searching in databases reporting deleterious and benign variants, and ID/ASD-associated genes. VOUS variants encompassing no genes or genes without any apparent brain expression/function were further investigated for potential long range position effects through involvement of Topologically Associating Domains (TADs), Lamina Associated Domains (LADs), and other chromatin activation signatures by using the web-based 3D Genome Browser and UCSC Genome Browser.

We identified non-benign CNVs in 314 patients. Among the pathogenetic and the probably pathogenetic variants (19%), we found CNVs spanning known ID/ASDs-associated genes, including two different de novo deletions and one duplication affecting genes whose mutations, recently identified by whole exome sequencing (WES) studies, were thought to act in ASDs with loss or gain of function mechanisms, respectively. We further identified 15 cases with de novo VOUS variants involving novel candidate genes and/or regions of regulatory expression of flanking ASD-associated genes as one deletion and one duplication spanning potential TAD boundaries and LADs.

The updating of diagnostic array-CGH CNVs in the light of new candidate genes and pathogenetic mechanisms allowed us to unravel complex cases, and revealing unexpected position effects.

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Possible position effect on *COL4A3BP* gene regulation in a family affected by neurological impairment

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We describe a 46,XX child aged 3 years, who came to our attention because of slight psychomotor delay (GQ=77), nystagmus, esotropia, and facial dysmorphisms, namely

brachycephaly, round face, and palpebral upslanting. Family history shows psychomotor retardation, mild school difficulties (IQ =67), and nystagmus in her older brother, partial cryptogenic epilepsy and esotropia in the mother, and Rendu-Osler-Weber Syndrome both in the father and in the brother. Array-CGH analysis (60K) identified a rare microdeletion of 70 kb in the child, in the mother and in the brother, mapping in 5q13.3 region (chr5:74822073-74892303). This deletion involves the *POLK* gene, not currently known as a disease gene. Interestingly, the deletion proximal breakpoint maps about 20 kb from the 5' UTR of *COL4A3BP* gene, which leads to mental retardation, autosomal dominant 34 (MRD34) [MIM:616351]. There is evidence of few cases with *de novo* missense mutations in *COL4A3BP* gene reported as pathognomonic for a phenotype characterized by psychomotor and global development delay, intellectual disability, epilepsy, craniofacial dysmorphisms, and skeletal features. The subsequent high resolution 1M a-CGH analysis finely maps the proximal deletion bkp upstream the *COL4A3BP* promoter, located in a region with several predicted regulatory elements (promoter region, enhancer and transcriptional elongation). In our hypothesis the lack of mentioned regulatory elements may compromise COL4A3BP gene expression by altering the gene regulatory domain. To confirm a position effect event and assess the pathogenic role of the identified CNV quantitative *COL4A3BP* gene expression analysis is ongoing.

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P09.041A

A novel loss-of-function mutation in HOXB1 associated with autosomal recessive hereditary congenital facial palsy in a large iranian family

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Introduction: Hereditary congenital facial palsy (HCFP) is a rare congenital cranial dysinnervation disorder, recognisable by non-progressive isolated facial nerve palsy (cranial nerve VII). It is caused by developmental abnormalities of the facial nerve nucleus and its nerve. So far, 4 homozygous mutations have been identified in 5 unrelated families (12 patients) with HCFP worldwide.

Materials and Methods: a large Iranian consanguineous kindred with 5 members affected by HCFP underwent thorough clinical and genetic evaluation. The candidate gene *HOXB1* was screened and analysed by Sanger sequencing. As in previous cases, the most remarkable findings in the affected members of the family were mask-like faces, bilateral facial palsy with variable sensorineural hearing loss, and some dysmorphic features.

Results: Direct sequencing of the candidate gene *HOXB1* identified a novel homozygous frameshift mutation (c.296_302del; p.Y99Wfs * 20) which cosegregated with the disease phenotype within the extended family.

Conclusions: Our findings expand the mutational spectrum of *HOXB1* involved in HCFP and consolidate the role of the gene in the development of autosomal recessive HCFP. Moreover, the truncating mutation identified in this family leads to a broadly similar resentation and severity observed in previous patients with nonsense and missense mutations. This study haracterises and defines the phenotypic features of this rare syndrome in a larger family than has previously been reported.

M. Vahidi Mehrjardi: None. **R. Maroofian:** None. **M. Dehghan Tezerjani:** None. **E. Zare Mehrjardi:** None. **H. Hozhabri:** None. **M. Jaafarinia:** None. **M. Dehghani:** None.

P09.042B

Congenital Variant of Rett Syndrome: Three siblings with *FOXG1* mutation due to maternal gonodal mosaicism in a Turkish family

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Background: Congenital variant of Rett syndrome is a severe neurodevelopmental disorder with features of classic

Rett syndrome, but earlier onset in the first months of life. It is the most severe form of atypical Rett syndrome, is generally caused by mutations in the *FOXG1* gene which is located on chromosome 14q12.

Case Presentation: A 8-year-old girl with had severe mental and motor retardation referred to our clinic because of two siblings also had similar findings. Delayed psychomotor development was noted at 6 months of age and she sit, crawl or walk all late. She could spaek only a few words. She also had bruxisim, tongue thrusting and sialorrhea. Brain MRI was normal except cavum septum pellucidum vergae. Karyotype was 46,XX and subtelomeric FISH was normal. Whole exome sequencing of patient reported with a heterozygous c.799G>A(p.Gly267Ser) variant at *FOXG1* gene. Variant classification was class 3 uncertain significance, according to in silico parameters variant was 4/4 damaging and prediction was disease causing. Due to clinical manifestations and heterozygous mutation confirmed by sanger sequence analysis at *FOXG1*, congenital variant of Rett syndrome (OMIM#613454) diagnosed. Parents were not consangious and they had also a 5-year-old girl and a 3-year-old boy have the same clinic and mutation. While father had no mutation at *FOXG1* gene mother had 19% mutation at DNA obtained from blood sample.

Conclusion: Totally three Congenital variant of Rett syndrome described clinically and genetically at this family. Possible segregation analyses stated that all siblings have disease due to maternal gonadal mosaicism.

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P09.043C

Intrafamilial variability of neuropsychiatric symptoms associated with the microduplication of chromosome 16p11.2

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Recurrent microduplications on chromosome 16p11.2 (OMIM #614671) have been implicated in childhood-onset developmental disorders including language delay, cognitive impairment, ADHD besides neuropsychiatric symptoms such as depression, anxiety, schizophrenia and bipolar disorder. We report a German family whose son had initially been investigated for connective tissue disease (ascending aortic aneurysm with 21 and joint laxity). Patient history was significant for learning disability but diagnosis of ADHD was only achieved at age 30. His father is being treated for depression and anxiety disorder and his younger sister has had a psychotic episode at age 18 and is currently on anti-depressant treatment. After NGS analysis for connective tissue diseases was normal in the proband, SNP array (Affymetrix CytoScan® HD) identified a heterozygous microduplication of 554 kb at 16p11.2. qPCR analysis confirmed segregation also for the father and sister. Among the 28 genes duplicated *KIF22* and *TBX6* are associated with joint laxity, for other candidate genes behavioral aberrations have been reported in mouse models. From those we selected *KIF22*, *DOC2A* and *SEZ6* and are performing qPCR studies in fibroblasts and iPSCs of the affected sister and unaffected mother. Our case demonstrates the broad intrafamilial spectrum of neuropsychiatric disturbances and adds data to the expression pattern of candidate genes contributing to the mental phenotypes of microduplication carriers. Interestingly, when comparing the phenotype to another family with a CNV associated with neuropsychiatric symptoms (16p13.2 deletion) affected women seem to be more vulnerable to later onset psychotic manifestations and carrier men rather to early onset neurodevelopmental disturbances.

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P09.044D

Dosage imbalance of the chromatin remodeler *CHD1L* is responsible for the neurodevelopmental and under/overgrowth phenotypes of the 1q21.1 CNVs

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Recurrent reciprocal 1q21.1 deletions and duplications have been found in individuals with syndromic autism. Variable phenotypes have been reported, including congenital heart defects, autism, schizophrenia, head circumference and height defects. The deletion is associated with microcephaly and short stature, whereas the reciprocal duplication is associated with increased risk of macrocephaly and carriers tend to be in the upper height percentiles, suggesting a possible undergrowth/overgrowth phenotype. We modeled the 1q21.1 duplication by overexpressing each of the eight 1q21.1 genes in zebrafish. Strikingly, we found that the overexpression of the chromatin remodeler *CHD1L* induces an increased number of both brain proliferating cells and post-mitotic neurons in the anterior forebrain at 2dpf, resulting in macrocephaly at later stages. Consistently,

suppression of the zebrafish ortholog of *CHD1L* by CRISPR/Cas9 led to a significantly decreased head size at 5dpf. We further evaluated whether *CHD1L* could be implicated in the growth abnormalities observed in 1q21.1 CNV carriers; its overexpression indeed led to a significant increase of the total body length and inter-somites distance at 5dpf. We also showed that the combinatorial overexpression of *CHD1L* and *GJA8*, another 1q21.1 gene, exacerbates the neurodevelopmental alterations induced by *CHD1L* overexpression alone, but does not impact further the body growth, suggesting a genetic interaction between *CHD1L* and *GJA8* on some, but not all phenotypic components. Our results suggest that *CHD1L* is a major contributor of the 1q21.1 CNV-associated neurodevelopmental phenotypes and indicate that *CHD1L* has a potential role in the control of human growth via an epigenetic regulatory mechanism.

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P09.045A

Malformations of the cerebal cortex: from targeted next generation sequencing to whole exome sequencing

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Cortical brain malformations (rare disorders of proliferation, neuronal migration or cortical organization) have been associated with mutations in a rapidly growing number of genes. This complicates molecular diagnostic by Sanger sequencing. Until 2015, we used a targeted Next Generation Sequencing (NGS) based work flow with a panel of 103 genes for routine diagnostic. Nowadays our flow is based on whole exome sequencing (WES) using a filter for a panel of 175 relevant genes. This approach has the advantage that novel genes can be easily included and expansion to full exome analysis is possible.

The WES work-flow involved the DNA enrichment using the Agilent SureSelect CRE Capture. The detected variants are filtered and annotated with the Cartagenia software and classified with Alamut Visual

DNA samples of 108 individuals were tested with the WES based panel. Eight patients (7,4%) received a direct diagnosis and 12 (11.1%) patients were solved after additional investigations. With the previous used targeted NGS approach in total 192 patients were tested with a diagnostic yield of 12,5%.

However, most of the identified alterations are variants of unknown clinical relevance. Despite the use of in silico prediction programs, usage of frequencies, evaluation of the conservation among species they cannot be judged without additional clinical information and feedback from the specialist.

In conclusion, the WES approach for malformations of the cerebal cortex is a powerful tool for DNA diagnostics. In order to increase the diagnostic yield of cortical brain malformations, close collaboration between laboratory and referring specialist is mandatory.

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P09.046B

***CYP2C19* and *CYP3A4* gene variants and schizophrenia in Armenian patients**

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Introduction: Genetic variations play an important role in antipsychotic drug treatment response in several mental disorders, including schizophrenia. Clinical studies suggested that antipsychotic drug metabolizing enzymes of cytochrome P450 family contribute to reduction of disease symptoms and manifestation of side effects. In this study we aimed to investigate the potential of schizophrenia with two single nucleotide polymorphisms (SNPs) of genes, coding CYP2C19 and CYP3A4 enzymes (*CYP2C19* rs4244285 and *CYP3A4* rs2740574, respectively).

Materials and Methods: Here patients with paranoid form of schizophrenia and healthy subjects of Armenian population were enrolled. DNA was isolated using salting out method with a simple introduction of chloroform step. Genotyping was performed using PCR-SSP. Distribution of genotypes corresponded to Hardy-Weinberg equilibrium. Statistical analysis was performed using Pearson's Chi-squared test.

Results: We found that genotypes and allele frequency of the *CYP2C19* gene rs4244285 polymorphism was equally distributed among the study groups (*CYP2C19* 681A allele frequency in cases vs. controls: 0.11 vs. 0.17, $p = 0.46$). The same applies for the *CYP3A4* rs2740574*G allele frequency (0.017 vs. 0.019, $p = 0.96$). Interestingly, the minor allele frequencies obtained significantly differ from those in 1000 Genomes that might reflect ethnic differences in the populations enrolled.

Conclusion: Despite this pilot study identified no association between schizophrenia and genetic variants within the *CYP2C19* and *CYP3A4* genes, further studies in large sample size and independent research centers are required to clarify these findings.Funding: the basic part of the Ministry of education and science of the Russian Federation, state task project No. 6.6762.2017/BT.

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P09.047C

A missense variant in *PER2* is associated with delayed sleep-wake phase disorder

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Delayed sleep-wake phase disorder (DSWPD) is a circadian rhythm sleep disorder, and is characterized by an inability to fall asleep until very late at night and awaken at a socially acceptable morning time. The pathogenesis of DSWPD is poorly understood. Recently, several large scale GWASs of chronotype have reported genetic variants associated with variation in chronotype. Several associated variants were located in genes related to circadian rhythms. This study was performed to identify variants associated with DSWPD from known circadian genes. We focused on low-frequency missense variants. We utilized data obtained from databases of genetic variations (whole exome-/ genome- sequencing). Candidates were extracted by integrating the data and *in silico* assessment. DNA samples from 236 patients with DSWPD and 1,436 controls were genotyped to examine whether the candidates are associated with DSWPD. A missense variant (p.Val1205Met) in *PER2* showed a significant association with DSWPD (minor allele frequency (MAF) of 2.5% in cases and 1.1% in controls, P=0.026, odds ratio = 2.32). In addition, MAF of the variant in 222 patients with idiopathic hypersomnia was significantly higher than that in 3,554 controls (MAF of 2.3% in cases and 1.1% in controls, P=0.038, odds ratio = 2.07). PER2 is noted for its major role in circadian rhythms. PER2 forms a heterodimer with CRY, and the heterodimer plays an important role in the regulation of the circadian rhythm. The p.Val1205Met substitution was located in the PER2 CRY-binding domain. The substitution could be a potential genetic marker for circadian rhythms and sleep phenotypes. (Grants: KAKENHI and AMED)

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P09.048D

A novel role for DNAJC12, a gene recently associated with hyperphenylalaninemia and early-onset dopa-responsive parkinsonism, in brain development

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Introduction: We recently described biallelic null-mutations in the *DNAJC12* gene in two probands of unrelated families with early-onset, dopa-responsive, and non-progressive parkinsonism. This gene encodes a member of DNAJ/Hsp40 family. It was suggested that DNAC12 interacts with the aromatic amino acid hydroxylases involved in the dopamine and serotonin metabolism. Despite this hypothesis, its function is still unclear. Understanding DNAJC12 physiological role could shed light on new pathways potentially involved in Parkinson disease pathogenesis and progression.

Materials and Methods: Immunofluorescence experiments were performed in HepG2 and in differentiated SH-SY5Y cell lines to assess DNAJC12 cellular localization and co-localization with potential cellular partners. To better characterize its function, the *DNAJC12*-zebrafish orthologue *(dnajc12)* has been cloned and the expression analyzed during embryogenesis. Gene functional ablation assays were carried out in zebrafish embryos using mRNA-specific antisense morpholino oligonucleotides. Histological analyses were performed to evaluate the effects of the loss-of-function approach.

Results: Our immunofluorescence experiments showed that DNAJC12 does not present a specific cellular localization but is present both in the cytoplasmic and the nuclear compartments. Concerning the *in-vivo* experiments in zebrafish, the dnajc12-morphants were characterized by a severe brain developmental phenotype. In particular, the morpholino-injected embryos displayed a marked expansion of the cerebral ventricles.

Conclusion: Our results suggest the existence of other unknown functions for DNAJC12 beyond dopamine and serotonin metabolism. Further studies will help shedding light on the specific role of this gene during early brain development.

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P09.049A

Identification of the genetic defect in patients with Dravet syndrome by a NGS gene panel for epilepsy

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Introduction: Dravet syndrome (DS, MIM 607208) is a severe early-onset infantile epilepsy that is mainly due to heterozygous mutations in *SCN1A*, and other genes. The application of a comprehensive NGS panel of genes related to epilepsy may allow identifying other genes and epileptogenic pathways involved in DS.

Materials and Methods: 125 patients with a presumptive diagnosis of DS were studied. The NGS panel *Epilepsy_v5.0*, with 425 genes related to epilepsy, was applied by Roche Nimblegen SeqCap EZ capture and Illumina NextSeq. The in-house bioinformatics analysis tool was applied to the alignment and annotation of variants. Filtering of variants, variant interpretation by ACMG/AMP and subsequent validation by Sanger sequencing or MPLA were performed.

Results: *Epilepsy_v5.0* applied over 125 DS patients allowed the molecular diagnosis of 47 patients carrying *SCN1A* pathogenic (P, 25), likely pathogenic (LP, 15) or variants of unknown significance (VOUS, 7). Moreover, 39 SD patients without mutations in *SCN1A* carry P variants (6), LP (2) or VOUS (31) in 35 additional genes that could explain their molecular defect.

Conclusions: 68.8% of the tested patients (86/125) presented variants in SCN1A and additional genes that could explain DS. The application of *Epilepsy_v5.0* has improved the mutational rate of *SCN1A* by Sanger sequencing and MLPA in DS patients, established in our laboratory in 44.3%. Genes identified in this study and not previously related to DS may open a new way for the molecular diagnosis and treatment of DS patients.

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P09.050B

Co-dysregulation of epilepsy genes and disrupted chromatin architecture in an iPSC-derived model of Dravet syndrome

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Introduction: Genetic epilepsies are devastating conditions for patients and their families. Existing anticonvulsant drugs do not provide satisfactory seizure control in one third of all patients. One approach to advance the development of novel antiepileptic drugs is to identify druggable molecular mechanism underlying the disease.

Materials and Methods: We established a disease model of SCN1A associated early onset epilepsy, i.e. Dravet Disease, using induced pluripotent stem cells (iPSC) from three patients with distinct and heterozygous SCN1A variants, and from three independent control individuals. The iPSCs were differentiated into cortical GABAergic interneuron-like cells over 65 days and subsequently analysed by whole-cell patch clamp recordings and RNA sequencing.

Results: Patch-clamp recordings showed decreased fast sodium currents in neuron-like cells from the three patients. Transcriptome profiling of neuron-like cells from Dravet patients revealed persistent upregulation of genes involved in chromatin remodeling. Additionally, several genes belonging to an epilepsy gene network were dysregulated supporting functional convergence for a group of genetic epilepsies.

Conclusions: Our data suggest chromatin remodeling to be perturbed in neurons with SCN1A variants from Dravet disease patients thus providing candidate pathways for the development of antiepileptic drugs.

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P09.051C

Expanding the clinical and molecular spectrum of DYRK1A-related disorder: report on novel mutations in three patients with syndromic intellectual disability, microcephaly, febrile seizures, distinctive facial dysmorphisms and cerebellar vermis hypoplasia

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Background: Haploinsufficiency of *DYRK1A* underlies a recognizable Intellectual Disability (ID) syndrome (MIM #614104). To date, about 50 patients have been reported with clinical features including microcephaly, intrauterine growth restriction, global developmental delay, ID, feeding difficulties, and distinctive facial dymorphisms. Other common finding include febrile seizures, and behavioral disturbances encompassing autism spectrum disorder, attention deficit disorder, and stereotypic movements. Aspecific brain malformations have been described, including enlarged ventricles, cortical atrophy, thin brainstem and thin corpus callosum.

Results: We report on three Italian patients heterozygous for previously unreported *de novo DYRK1A* mutations identified by exome sequencing. The three variants were predicted to elicit a disruptive effect, being frameshift/nonsense changes. In one subject, the impact on transcript processing was confirmed experimentally.The three patients presented clinical features within the phenotypic spectrum associated with *DYRK1A* mutations, including intrauterine growth restriction, microcephaly, severe speech impairment, ID, feeding difficulties, febrile seizures, behavioral issues and suggestive facies (prominent metopic appearance, deep set eyes, blepharophimosis, microretrognathia). Of note, all patients presented brain abnormalities, including cerebellar vermis hypoplasia, which have not previously been reported to occur in individuals with *DYRK1A* mutations.

Conclusion: Our findings provide further information on the clinical phenotype spectrum associated with truncating mutations in *DYRK1A*, adding cerebellar vermis hypoplasia as a previously unappreciated feature of *DYRK1A* haploinsufficiency.

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P09.052D

The KIAA0319 dyslexia susceptibility gene presents a highly specific expression pattern during the development of different organs & plays a role in cytoskeleton dynamics

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The association between the KIAA0319 gene and dyslexia was reported almost 15 years ago. While the association has been consistently replicated, KIAA0319 function remains poorly understood. Initial characterizations showed a specific expression in the human developing cortex and in utero shRNA experiments in rats suggested a role in neuronal migration. Conversely, recent studies in mice reported effects in the auditory system but not in neuronal migration. To further elucidate the function of KIAA0319 we used a range of approaches. Gene expression analysis in zebrafish revealed a specific pattern restricted to particular developing structures, confirming a role in the brain and in the auditory system as well as in the visual system and the notochord. The dyslexia associated genetic variants reside in regulatory sequences and might therefore affect this tightly regulated spatial/temporal pattern. To investigate the function of KIAA0319, we generated and characterised stable cellular knockouts with different assays. These included the newly developed Elastic Resonator Interference Stress Microscopy (ERISM) system which allows the study of mechanical forces, such as cell-substrate interactions, at single cell level. This approach shows that KIAA0319 plays a role in cell motility, migration and attachment, three processes mediated by the cytoskeleton. Taken together, these data support a role for KIAA0319 in cytoskeleton dynamics, consistent with an involvement in neuronal migration. However, such role is likely to extend beyond brain development and contribute also to the development of sensory organs and the notochord. Supported by the Royal Society, Northwood Trust, EPSRC and the ERC.

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P09.053A

Dopaminergic dysfunction in a rat model for DYT6 dystonia

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Mutations in THAP1 (thanatos-associated protein domain-containing apoptosis- associated protein 1) cause autosomal dominant primary dystonia 6 (DYT6 dystonia). To date, more than eighty different mutations / variations have been identified in the THAP1 gene in different ethnic populations with unknown pathogenic mechanism. Recent studies of Thap1 mouse models showed a delay in myelination or

dysfunction of pathways related to eIF2α Signaling and mitochondrial dysfunction. However, the neuropathological and neurophysiological changes in rats due to THAP1 dysfunction are unknown. Using the CRISPR/cas9 technique, we generated a novel model for THAP1 dystonia in a different species. As observed in THAP1 mouse models homozygous Thap1 knock-out rats are not viable. RNA-seq and protein analysis in heterozygous KO rats revealed expression changes of genes involved in doparminergic functios. Electrophysiological studies in the striatum revealed abnormal firing frequency in Thap1 heterozygous knock-out rats stimulated with amphetamine. Taken together, our novel THAP1 dytonia rat model showed a dystonia related phenotype associated with dopaminergic dysfunction.

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P09.054B

A novel de novo nonsense mutation in *STXBP1* found in child diagnosed with early infantile epileptic encephalopathy type 4

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Background: Early Infantile Epileptic Encephalopathy Type 4 (MIM:612164) is autosomal dominant disease caused by heterozygous mutation in the *STXBP1* gene. Here we present the clinical case report. The boy (birth year 2013): at the 3rd week of life a single focal seizure attack was appeared. In 4 months resumed seizures (complex partial and secondary generalized tonic-clonic). In 5 months there were seizures on the type of infantile spasms. At 2014 we have performed the molecular-genetic study.

Methods and Results: NGS sequencing (454 GSJunior; NimbleGen SeqCap target enrichment; testing mutations for genes, associated with epileptic encephalopathy): in *STXBP1* gene we have found heterozygous mutation c.1235_1236delCC (NM_003165.3) or, using uniprot canonical transcript, c.1038_1039delCC (CCDS35146.1/ CCDS6874.1). This two-nucleotide deletion results to frame-shift joined with nonsense mutation, changing protein sequence: "...THLHL..." => "...TPAPC*". Further clinical exome analysis (Illumina) did not revealed any other candidate-mutations. Clinical picture of disease allowed to assume de novo mutation. Data analysis was performed using in-house pipelines. In 2017 molecular genetic study for trio (proband with parents) performed at Birmingham Women's Hospital (England) confirmed de novo mutation in *STXBP1* for proband.

Conclusions: Presently, the boy 3y/o is under supervision and drug therapy; he has some temporal delay of psychomotor and speech development. The authors are grateful to MD Zhilina S. for clinical genetics consulting. The research was supported by the Department of Health of Moscow (project 2014-2015).

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P09.055C

Multi-gene panel testing improves diagnosis in Brazilian patients with Early-Onset Epilepsy

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Introduction: Early-onset epilepsy (EOE) is characterized by seizures of variable intensity, cognitive impairment and unusual behavior. Approximately twelve forms of epilepsy have genetic etiology, however, in more than a half of the EOE patients, the basis remains unknown. Thus, an unequivocal molecular diagnosis in these patients is essential to develop targeted-disease therapies.

Material and Methods: We studied 147 patients with seizures and/or myoclonus whose started between 2 and 4 years of age using a customized panel (Agilent SureSelect technologies / MiSeq Illumina, CA). A total of 51 genes associated to epilepsy in previous publications were selected for investigation. The analysis was performed using VariantStudio (Illumina, CA) and SureCall (Agilent, CA).

Results: Our results revealed 72 patients with 113 relevant variants in 42 different genes, highlighting ATM, CLN6, MSFD8, MECP2 and TPP1 genomic variants. Of the 113 relevant variants detected by NGS panel, 20 were classified as pathogenic, 31 likely pathogenic, 41 were a variant of uncertain significance (VUS) and 21 likely benign. The most frequent variant was single nucleotide variants (SNVs) and InDels, being 91 in heterozygosis e 22 in homozygosis.

Conclusions: A total of ~49% of patients obtained a potential genetic diagnosis evidencing the efficiency of our customized panel, which allows a high detection rate with a lower cost than a whole exome. A customized multi-gene panel allows detect genetic variants and also could further improve genetic diagnosis in patients with early-onset Epilepsy.

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P09.057A
Diagnostic yield of aCGH and NGS gene panels in epilepsy

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Introduction: genetic testing is a very powerful tool for the diagnosis of epilepsy. Copy-number variations (CNVs) or point mutations cause epileptic disorders or predispose to such heterogeneous pathology. Detection of CNVs require one method such as microarray CGH (aCGH), while point mutations within related genes could be detected by next generation sequencing technologies (NGS). We performed a retrospective cohort study, comparing the diagnostic yield of aCGH and NGS gene panels in epilepsy.

Material and Methods: we performed a retrospective cohort study of a series of neuropediatric patients with seizures as the main symptom of epilepsy. In 158 cases, aCGH was performed, while 231 cases were studied by Illumina targeted-exome sequencing and specific NGS panels.

Results: From the 158 cases studied by aCGH we detected pathogenic or likely pathogenic CNVs in 26 (16.4%) patients. As for NGS panels, we identified deleterious mutations in 27 (17.7%) cases with 46-gene panel and 22 cases (27.8%) with 543-gene panel.

Conclusions: we achieved an effective diagnostic two-step method for neuropediatric patients with epilepsy. It consists of aCGH analysis followed by the analysis of specific NGS panels. Diagnostic rate increased by 35% due to the application of NGS panels, in an efficient, accurate and cost-effective strategy.

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P09.058B
Gain-of-function variants in the *CASR* gene in genetic generalized epilepsy

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Introduction: Genetic generalized epilepsies (GGE) are a common form of human epilepsies with substantial genetic basis to their etiology. The *EIG8* (3q13.3-q21) locus for GGE was identified in a three-generation family from south India (Kapoor et al. *Ann Neurol* 2008).

Methods: Sequence analysis of the 64 Mb haplotype at 3p14.2-q21, being shared by all affected members of the family was conducted by whole-exome sequencing (WES). *CASR* was sequence analyzed in unrelated 480 GGE/JME patients and 504 control chromosomes. Further, the functional implications of rare non-synonymous mutations identified were evaluated on the CASR-regulated signaling pathways.

Results: In WES analysis, five disease co-segregating rare variants in the *EPHA6*, *ABI3BP*, *KIAA1407*, *IQCB1* and *CASR* genes were found. Of these, c.2693G>A (p. Arg898Gln) in *CASR* fulfilled the criteria of being a causative mutation. *CASR* sequence analysis, identified five additional rare non-synonymous mutations, p.Glu354Ala, p. Asp433His, p.Ser580Asn, p.Ile686Val and p.Ala988Val in 14 unrelated GGE/JME patients. In cell signaling assays, the mutant CASR receptors exhibited leftward shifts in the dose-response curves showing an enhanced responsiveness to extracellular calcium concentrations as compared to the wild-type receptor, thereby suggesting, activating nature of these variants.

Conclusion: Our findings indicate a role for CASR in predisposition to GGE/JME based on evidence provided at the gene- and variant-level. Based on observed enhanced calcium responsiveness of CASR alleles in the cell signaling assays, we propose that gain-of-function effects of these mutations may alter CASR-regulated functions in the brain and contribute to pathophysiology of epilepsy.

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P09.059C

Detection of copy number variations in epilepsy using exome data

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Epilepsies are neurological disorders and genetic factors contribute to their pathogenesis. Copy number variations (CNVs) are increasingly recognized as an important etiology of many human diseases including epilepsy. Whole exome sequencing (WES) is becoming a standard tool for detecting pathogenic mutations and has recently been applied to detecting CNVs. Here, we analyzed 294 families with epilepsy using WES, and focused on 168 families with no causative single nucleotide variants (SNVs) in known epilepsy-associated genes to further validate CNVs using two different CNV detection tools using WES data. We confirmed 18 pathogenic CNVs, and two deletions and two duplications at chr15q11.2 of clinically unknown significance. Of note, we were able to identify small CNVs less than 10 kb in size, which might be difficult to detect by conventional microarray. We revealed two cases with pathogenic CNVs that one of the two CNV detection tools failed to find, suggesting that different CNV tools are recommended to increase diagnostic yield. Considering a relatively high discovery rate of CNVs (18 out of 168 families, 10.7%) and successful detection of CNV with <10 kb in size, CNV detection by WES may be able to surrogate, or at least complement, conventional microarray analysis. Acknowledgements: Nakashima M, Kato M, Heyman E, Inui T, Haginoya K, Watanabe S, Chiyonobu T, Morimoto M, Ohta M, Kumakura A, Kubota M, Kumagai Y, Hamano S-I, Lourenco CM, Yahaya NA, Ch'ng G-S, Ngu L-H, Fattal-Valevski A, Hubshman MW, Orenstein N, Marom D, Cohen L, Goldberg-Stern H, Nakajima H, Saitsu H, Miyatake S.

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P09.060D

The use of Next Generation Sequencing for the diagnosis of early onset epilepsy and epileptic encephalopathies

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Epilepsy comprises a wide range of etiologically very heterogeneous clinical conditions ranging from benign forms to treatment-refractory progressive encephalopathies, which clinical features, seizure type, age of onset, electroencephalographic features and response to anti-epileptic drugs are very diverse and may vary over time. At present, targeted sequencing of genes associated with genetically heterogeneous conditions seems to be the elective choice for early and efficient etiological diagnoses. 155 individuals have been subjected to NGS investigation, using a customized panel of 31 genes, on the Ion PGM™ sequencing platform. Sequence variants were interpreted according to the ACMG guidelines. The average sequencing depth of coverage was 331.6X, with 97.6% of the reads on-target and 93.2% of reads uniformity. On average, 114 variants per patient have been detected. In the overall, we were able to identify disease-causing variants in 27 individuals (17.4%) although, since the panel mainly targeted EIEE/early epilepsy genes, the diagnostic yield stratified according to age of seizure onset resulted 27% in cases with onset within 6 months of life, 22.7% within 12 months of life, 19.2% in cases with seizure onset before 24 months of life. Out of 27 pathogenic/likely pathogenic variants 19 were found to alter five voltage-gated ion channels: *SCN1A* (7), *SCN2A* (4), *SCN8A* (4), *KCNQ2* (2) and *HCN1* (2). Our work further strengthens the importance of a careful phenotype characterization coupled with the power of the NGS technology and indicates in a subset of few genes the major players for epilepsy with very early onset.

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P09.061A
De novo variants in neurodevelopmental disorders with epilepsy

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Epilepsy is a frequent feature of neurodevelopmental disorders (NDD) but little is known about genetic differences between NDD with and without epilepsy. We analyzed de novo variants (DNV) in 6753 parent-offspring trios ascertained for different NDD. In the subset of 1942 individuals with NDD with epilepsy including 529 individuals with epileptic encephalopathy, we identified 33 genes with a significant excess of DNV. Of these, *SNAP25* and *GABRB2* had previously only limited evidence for disease association. Joint analysis of all individuals with NDD also implicated *CACNA1E* as a novel disease gene. Comparing NDD with and without epilepsy, we found missense DNV, DNV in specific genes, age of recruitment and severity of intellectual disability to be associated with epilepsy. 24 routinely used diagnostic panels would only have detected on average 59% of DNV in the 33 genes with exome-wide DNV burden. We further found low evidence for disease association for genes frequently used on diagnostic epilepsy panels. 5% of DNV in our study were in eight genes for which we could confirm therapeutic consequences with established evidence-based medicine criteria emphasizing the benefit of accurate genetic diagnosis in NDD with epilepsy.

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P09.062B
Functional characterisation of novel SCN1A mutations in epileptic disorders

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Mutations in *SCN1A*, the gene encoding voltage-gated sodium channel $Na_V1.1$, cause a spectrum of epilepsy disorders that range from genetic epilepsy with febrile seizures plus to severe disorders such as Dravet syndrome. To date, more than 1,250 mutations in *SCN1A* have been linked to epilepsy but only a small number of them has been functionally characterised. We identified several novel mutations (p.E78D, p.D249E, p.W384X, p.E777K, p.T1923I) in *SCN1A* gene in Slovak epilepsy patients, which were subsequently subjected to functional characterisation in heterologous expression system. *EGFP* gene was inserted to pCDM8-h$Na_V1.1$ to visualise the eukaryotic cells expressing protein of interest. All identified mutations were introduced to pCDM8-h$Na_V1.1$-EGFP construct and were propagated in TOP10/P3 *E.Coli* grown at 28°C to minimize the rearrangements. The entire coding sequence was sequenced after each propagation. The pathogenic effect of each mutation on protein function was tested in transiently transfected HEK293T cells by whole-cell patch clamp configuration. Cells were also transfected with each of accessory β subunits to test whether mutant channels can be rescued by molecular interactions with these modulatory proteins. Finally two antiepileptic drugs, phenytoin and carbamazepine, and an antiarrhythmic drug, mexiletine, which probably act by stabilizing the correct folding conformation were tested, if they can prevent the degradation of the mutant protein and reduce the loss of function effect. These findings may contribute to the understanding of mechanisms of the epileptogenesis and to the effective therapy.

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P09.063C

Monoallelic expression of *TTR* gene as a contributor to phenotypic variability of TTR-related amyloidosis

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Monoallelic gene expression is a phenomenon in which only one allele from a homologous pair is transcribed. Transthyretin (TTR) amyloidosis is an autosomal dominant systemic disorder caused by mutations in the *TTR* gene. Markedly different penetrance according to the gender of the transmitting parent as well as a variable manifestation between monozygotic twins was observed in Bulgarian families. The aim of the present study was to better understand the difference in the disease penetrance by evaluating the mutant versus wild type transcripts. The RNA was extracted from plasma and urine and the *TTR* RT-PCR products were sequenced by Sanger. Apart from the expected traditional biallelic transcription a monoallelic expression signature of only mutant or only wild type alleles was observed. For some patients tissue-specific transcription profile was detected, which corresponds to multiple tissues and organs involvement in the disease manifestation. Based on our results we propose a model of natural selection, which includes age-related allele suppression: predominant expression of a wild type allele (at an early age) and mutant allele (at the process of ageing). Different regulatory mechanisms at molecular level (histone modifications, chromatin remodelling, transcription factors, and epigenetic alterations) might be involved and combined in different manner in different individuals, which explains interfamilial differences and phenotypic differences in monozygotic twins with identical genotypes. Further studies on monoallelic expression of the *TTR* gene will facilitate a better understanding of the *TTR* gene transcriptional regulation. Acknowledgement: The study was supported by Pfizer: Grant №WI220557/15.11.2016.

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P09.064D

Assesment of candidate genes in patients with frontotemporal lobar degeneration spectrum: preliminary findings

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Frontotemporal lobar degeneration (FTLD) is a heterogeneous disorder group associated with degeneration in the frontal/temporal lobes of brain. This study is aimed to determine the frequencies of mutations in *MAPT, PGRN,CHMP2B,VCP,TARDBP* and *FUS* genes, which are considered as the main genetic causes of FTLD in Turkish population and to investigate the genotype-phenotype correlations in cases with pathogenic/ likely-pathogenic variants. The exon/exon-intron junctions for related genes in gDNAs of 100 FTLD cases and 100 age-matched controls were sequenced by using IonTorrentS5, then analyzed with bioinformatics pipeline. NGS results were confirmed by the Sanger Sequencing and are shown in table without variants considered as benign.

It was identified 2 novel variants in *MAPT* and *CHMP2B* genes that are intronic and missense, respectively. Additionally, 2 missense and 2 frameshift mutations were detected in *GRN* and 1 missense mutation in *TARDBP*. Interestingly c.759_760delTG *GRN* and c.389A>G *CHMP2B* variants were identified in the same patient who has died a year after the diagnosis. Segregation studies of family members are in progress.

This study indicates that GRN mutations are more common causative genetic factors for FTLD (%4) and this ratio is% 12 in cases with positive family history. To our knowledge, this is first report evaluating the genetic backround in Turkish FTLD. This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK1001-114S346)

Table: NGS Results

Genes	cDNA rs ID	Protein	*In siliko* prediction	MAF %	Subclass of FTD	Age of Onset	Family Hist	Classification
GRN	c.415T>C rs763841075	p. Cys139Arg	PD/Del/DC	0.018	bvFTD	55	+	Pathogenic**
GRN	c.430G>A rs200591137	p. Asp144Asn	PD/Del/DC	0.00081	SD	59	+	VUS***
GRN	c.102delC rs63751073	p. Gly35Glufs	DC	NA	bvFTD	58	+	Pathogenic**
GRN*	c.759_760delTG rs63751035	p. Cys253Terfs	DC	0.00041	bvFTD	56	+	Likely Pathogenic***
CHMP2B*	c.389A>G **Novel**	p. Lys130Arg	PD/Tol/DC	NA	bvFTD	56	+	VUS***
TARDBP	c.1213A>G rs762209110	p. Met405Val	PD/Tol/DC	NA	bvFTD	72	–	VUS***
MAPT	c.1828-3A>C **Novel**	p.?	–	NA	bvFTD	42	+	VUS***

MAF: Minor allel frequency from gnomAD (The Genome Aggregation Database),

In silico prediction: Polyphen, SIFT, Mutation Tester respectively

PD: Probably Damaging, Del: Deleterious, DC: Disease Causing, Tol: Tolareted

*Same patient

**Defined in HGMD (Human Gene Mutation Database)

*** According to ACMG (American College of Medical Genetics and Genomics) criteria

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P09.065A

Role of mitochondrial DNA variants in the development of FXTAS

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Introduction: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder that appears in at least one-third of adult carriers of a premutation (55-200 CGG repeats) in the *FMR1* gene. Although several studies have described the impairment of mitochondrial function in FXTAS patients, to our knowledge there are no data regarding the involvement of mtDNA in FXTAS. The aim of this study is to elucidate the role of mtDNA variation in the pathogenesis of FXTAS.

Materials and Methods: Two independent sets of *FMR1* premutation carriers were recruited. In the first set (13 FXTAS and 13 no-FXTAS) the entire mitogenome was sequenced using massively parallel sequencing technologies. In the second set (39 FXTAS and 67 no-FXTAS), mitochondrial haplogroups were determined.

Results: We identified haplogroup T differentially enriched in *FMR1* premutation carriers and significantly underrepresented in FXTAS patients. Analysis of mtDNA sequences revealed an association between disease and the burden of heteroplasmic variants and their distribution. The FXTAS group presented 3-fold more low-level heteroplasmic variants in compromised regions of the mitochondrial genome.

Conclusions: Our results suggest that haplogroup T might be a potential protective factor for FXTAS. In addition, FXTAS individuals accumulate higher rates of heteroplasmic variants in compromised regions of the mitochondrial genome. These results may explain, in part, the role of mtDNA in the development of FXTAS. This work was supported by the Instituto de Salud Carlos III (PI12/00879; PI17/01067), co-financed by Fondo Europeo de Desarrollo Regional (FEDER) "una manera de hacer Europa" and AGAUR (2014SGR603; 2014SGR1420; 2017SGR1134).

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P09.066B

Decreased mitochondrial DNA copy number is associated with clinical manifestations of FXTAS

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Introduction: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder with reduced penetrance that appears in adult *FMR1* premutation carriers (55-200 CGGs). There are several studies supporting a role for mitochondrial dysfunction in the pathogenesis

of FXTAS. However, the mtDNA copy number has been poorly studied.

Material and Methods: mtDNA copy number was studied in multiple tissues from FXTAS patients and matched control subjects (post-mortem human brains, blood samples and skin fibroblasts cultures). The results were compared to age-matched controls. Digital droplet PCR and Real Time quantitative PCR was performed was used to determine the mtDNA copy number.

Results: The analysis of mtDNA levels in human tissues evidenced reduced mtDNA content in cerebellar vermis, dentate nucleus, parietal and temporal cortex areas. The fact that no mtDNA copy number alteration was detected in any other brain regions or in any other tissues analyzed, suggests that its potential effect is restricted to clinically relevant regions explaining why FXTAS clinical manifestations are associated with gait ataxia and tremor.

Conclusions: Our study indicates that reduced mtDNA copy number is restricted to the affected brain tissue and provides new insights into the role of mitochondrial dysfunction in the pathogenesis of FXTAS. Acknowledgements: This work was supported by the Instituto de Salud Carlos III (PI17/01067), co-financed by Fondo Europeo de Desarrollo Regional (FEDER) "una manera de hacer Europa" and AGAUR from the Autonomous Catalan Government (2017 SGR1134). The CIBER de Enfermedades Raras is an initiative of the Instituto de Salud Carlos III.

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P09.067C

Low Dose Dexmethasone Decrease the Level of ATG5 in SH SY5Y cell line

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Introduction: Glucocorticoids (GCs) have a significant role in the adaptive response of the brain to stress. Increasing evidence has demonstrated that an increase of GC levels may induce neuronal cell death via apoptotic pathways. In the present study, we aimed to investigate whether the Dexmethasone, a synthetic glucocorticoid, at physiologic dose plays a role in unfolded protein response induced by Endoplasmic Reticulum Stress and Endoplasmic Reticulum Stress-Mediated Autophagy in neuron like cell line (SH-SY5Y).

Materials And Methods: SH-SY5Y cells were treated with 100ng/ml dexamethasone at acute stress dose for 4 hours. After RNA isolation and cDNA synthesis, Real-time PCR reactions were performed for GRP78, ATF4, XBP1 and ATG5 genes.

Results: We found that the expression level of Autophagy related 5 (*ATG5*), which has been previously characterized as a protein required for autophagy, was significantly decreased in SH-SY5Y cell lines that were exposed to Dexmethasone. However, we did not find any differences between genes (GRP78,ATF4,XBP1s) which are key players of unfolded protein response.

Conclusion: Relationship between decreased autophagy and neurodegenerative diseases in neuronal cells has been well studied. For this reason, our result may suggests that low dose of GCs can contribute to the pathogenesis of the neurodegenerative diseases through decreasing the expression of *ATG5*. Supported by CUBAP/TSA-2017-8116.

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P09.068D

Genetic analysis in GLUT1 deficiency syndrome

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Introduction: GLUT1 deficiency syndrome is defined as a metabolic encephalopathy that usually caused by pathogenic variations in the *SLC2A1* gene. The aim of the study is to determine the presence of single nucleotide and copy number changes in *SLC2A1* gene.

Method: In this study, all of the 10 exons in 13 patients with GLUT1 deficiency syndrome were sequenced by the Sanger method. Results were analyzed as *in-silico* with various bioinformatics tools, phenotype-genotype correlations were revealed. According to the ACMG Standards and Guidelines published in 2015, necessary to demonstrate that family members have blood connections with the patient for the confirmation of *de novo* variants. For this reason, SNP Array analysis was performed for 2 patients and their family members. In 7 patients who do not have any variation in Sanger analysis, quantitative real-time PCR was applied to each of the 10 exons in the *SLC2A1* gene to determine the presence of copy number variations.

Result: Sanger sequencing method, 8 variants were identified. 2 of them are novel and *de novo* variants. When

examined via databases, one of the novel and *de novo* variants is a splice-side variant, the other is a frame shift variant, and the phenotype effects were assessed as pathogenic by *in-silico* tools. qRT-PCR calculations for CNV detection are still ongoing.

Conclusion:%90 of individuals with Glut1 DS caused by *de novo* heterozygous variants in *SLC2A1* gene. Screening of this gene is important for understanding genetic basis of the disease and providing genetic counselling for patients.

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P09.069A

An activating *GNAO1* mutation causing refractory chorea suppressed by folinic acid therapy

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Introduction: There is no effective treatment for *GNAO1*-related movement disorder (MD). Here, we describe the novel use of folinic acid to control MD in a patient who carried an activating *GNAO1* pathogenic variant.

Materials and Methods: The patient is a 13-month-old Chinese girl who presented with global delay and dystonia. She had unremarkable birth history with no family history of consanguinity or neuromuscular disorders. She had poor truncal tone and persistent fisting at 5 months. At age one she could only vocalize. Physical examinations showed axial hypotonia with extremity hypertonia and brisk jerks. Biochemical and imaginings findings were unremarkable. Subsequent genetic analysis revealed a *de novo* heterozygous activating pathogenic variant, NM_020988.2 (*GNAO1*):c.736G>A; p.Glu246Lys.

Results: Patient's MD remained intractable. It persisted throughout the day, and only temporarily ceased during sleep. She was unresponsive to combined treatment, i.e. risperidone, nitrazepam, tetrabenazine and clonazepam, and required deep sedation with midazolam infusion. Folinic acid was administered for suspected secondary cerebral folate deficiency. Surprisingly, there was a significant reduction in MD within 2 days with improvement in awareness and motor functions (video). Pre-treatment CSF 5-methyltetrahydrofolate (MTHF) level was normal, measuring 75 nmol/L (reference interval: 40-128). Her MD remained well controlled with folinic acid (75 mg daily), low dose nitrazepam, carbamazepine and risperidone.

Conclusions: This is the first reported case of successful pharmacological control for intractable MD in a patient with an activating *GNAO1* mutation. A new inhibitory mechanism between folate and GNAO1 signaling was suggested by the clinical findings.

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P09.070B

Association of Haptoglobin-1 allele with Autism

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Gene-environment interaction, through abnormal intestinal adsorption, has been proposed as possible mechanism for autism pathogenesis in those patients lacking of causative genetic variants. Haptoglobin (HP) is a haemoglobin binding and acute-phase plasma protein, encoded by two co-dominant alleles, HP-1 and HP-2, producing pre-HP-1 and pre-HP-2 proteins that mature in HP-1 and HP-2, respectively. HP-2 allele contains a 1.7Kb tandem duplication that includes two extra exons with respect to HP-1.

Endogenous pre-HP-2 protein deregulates intestinal tight-junctions through EGFR and PAR2 activation, increases intestinal permeability and has been associated with autoimmune and inflammatory diseases as well as with psychiatric conditions.

Since the association between *HP* alleles and autism has never been investigated, we genotyped, by PCR analysis, *HP* in a cohort of Italian patients with autism ($n = 406$) and in controls ($n = 367$). The aim was to evaluate the possible role of HP-2 in enhancing macromolecular intestinal trafficking in these patients.

Contrary to what we expected, HP-1 allele distribution was different between patients and controls (36.3% and 29.4%, respectively) and significantly associated with autism ($P=0.0041$).

Since a subgroup of patients and controls have already been genotyped by Illumina Human Omni-15-8 v.1.0 and Affy-6.0 chips, respectively, we are trying to impute *HP* alleles from flanking SNP haplotypes. HP alleles will therefore be predicted in publicly available large cohorts of patients with autism.

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P09.071C

Retrospective study of symptomatic carriers of an Intermediate Allele (IA) in Huntingtin (HTT) gene

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Introduction: Huntington's Disease (HD) is an autosomal dominant neurodegenerative disorder characterized by uncontrollable movements (chorea) and subtle changes in cognition and behaviour caused by an expansion of CAG repeats (n≥36) in Huntingtin (*HTT*) gene. In this study we focus on symptomatic patients, carriers of intermediate alleles (IAs; n = 27-35), in an attempt to describe a possible phenotypic association.

Methodology: We reviewed the available data (phenotype and genotype) of symptomatic patients referred for HD genetic testing. Frequencies of IAs were compared with the general population. Clinical symptoms of IAs carriers were classified in three groups: motor, cognitive and behavioural.

Results: Frequency of IAs was significantly higher (X^2=6.77, p = 0.01) among symptomatic patients (3.69%) when compared to individuals of the general population (2.12%). Family and clinical data were available in 73 IA-symptomatic patients: 64.38% had 29-27 CAG repeats and 35.61% had 30-35 CAG repeats. Mean age at diagnosis was 61.27 (n = 71, SD=20.1), with signs starting 3.27 years earlier (n = 22, SD=2.46). Symptoms included abnormal movements, chorea, ataxia, dyskinesia, cognitive impairment and anxiety & depressive disorder. When classified in groups, 86.44% showed alterations in movement, 23.73% in cognition and 32.2% in behaviour (n = 59). A positive family history could be confirmed in 21% of patients.

Conclusion: Non-HD symptomatic patients seem to be more likely to carry an IA. However, except for a late age of onset of symptoms, they do not show a common recognizable phenotype. Further follow-up studies of IAs carriers may help understand the possible effect and penetrance of these alleles.

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P09.072D

Leucocyte Telomere Length in Huntington's Disease. Preliminary data

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Introduction: Huntington's disease (HD) is an autosomal dominant, fully penetrant, neurodegenerative disease caused by an expanded CAG repeat in the first exon of the HTT gene. The onset of symptoms most commonly occurs at midlife and inversely correlates with the CAG repeat expansion. However, age of clinical onset, progression rate, and severity of symptoms can vary between individuals. Leukocyte telomere length (LTL) has been widely investigated in neurodegenerative diseases such as Alzheimer's and Parkinson diseases, but very few data on LTL in HD have been reported. In the present preliminary study, we investigated the relationship between LTL and HD development, including premanifest and symptomatic HD patients.

Methods: LTL (T/S ratio) was measured in HD patients (n= 46) or pre-HD (n= 31), compared with LTL of age-matched controls (n= 60).

Results: significant LTL differences among controls, pre-HD and HD subjects were observed (p < 0.0001), with mean LTL values in the following order: HD patients < pre-HD < controls. After adjusting LTL for age, the differences in LTL across the three groups remained highly significant (p < 0.0001).

Conclusion: current data indicate that shortened LTL are observed in HD patients as found in other neurodegenerative disorders. The analysis of LTL in pre-HD patients (never examined to date) suggests that a progressive

telomere erosion may occur in the pre-manifest stage. The possible use of LTL as biomarker of disease progression is discussed.

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P09.073A

ANGPTL6 and Familial Susceptibility to Intracranial Aneurysm

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Intracranial aneurysms (IA) are acquired cerebrovascular abnormalities characterized by a localized dilation and wall thinning in intracranial arteries. The main IA complication is the rupture, resulting in subarachnoid haemorrhage and possibly leading to severe outcome. IA pathogenesis is still largely unknown, with no reliable risk marker available so far. We have recently identified one rare nonsense variant (c.1378A>T) in the last exon of *ANGPTL6* (Angiopoietin-Like 6) shared by the 4 tested affected members of a large pedigree with multiple IA cases. Since *ANGPTL6* encodes a circulating pro-angiogenic factor mainly secreted from the liver, we showed a 50% reduction of ANGPTL6 serum concentration in individuals heterozygous for the c.1378A>T allele compared to relatives homozygous for the normal allele, probably due to the non-secretion of the truncated protein produced by the c.1378A>T transcripts. By sequencing *ANGPTL6* in additional index cases with familial IA, we detected a significant enrichment in rare coding variants within this gene among 95 affected subjects compared to a reference population of 404 individuals with French ancestry. We observed a higher rate of individuals with a history of high blood pressure among affected *versus* healthy individuals carrying *ANGPTL6* variants, suggesting that ANGPTL6 could trigger cerebrovascular lesions when combined with other risk factors such as hypertension. Altogether, our results indicate that rare coding variants in *ANGPTL6* are causally related to familial forms of IA. We have now generated the knock-in mouse model for the ANGPTL6 c.1378A>T variant: the pathophysiological consequences of this truncating substitution are currently under further investigations.

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P09.074B

Joubert Syndrome:New genes described! A new allelic phenotypes achieved!

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Introduction: Joubert syndrome (JS) is characterized by hypotonia, ataxia, psychomotor delay, 'molar tooth sign'.JS is clinically and genetically heterogeneous.

Methods: NGS and array-CGH were used.An attempt of genotype-phenotype correlation has been created. All patients had dysmorphism, developmental delay and 'molar tooth sign' in MRI. *Case1*:7 year-old patient had hypotonia, microphtalmia, pytosis,aganglionic colon.Homozygous mutation (p.Arg563His) in *INPP5E* gene was detected. *Case2*:16 month-old patient had lobule tongue,bifid uvula, short thorax,tetramelic postaxial polydactyly.Homozygous 2-bp deletion in *IFT80* gene was detected. *Case3*:8 day-old baby had cleft palate, coloboma and pytosis.Array-CGH showed microdeletion of 136 kb at 16q22.1(This region included a PDPR gene). *Case4*:12 year-old patient had sleep disturbance, nephronophthisis and coloboma.Genetic analysis revealed compound heterozygous mutation c.6012-2A>G in splice site and c.5668 G>A (p.Gly1890X) in *CEP290* gene. *Case5*:3 year-old patient had breathing abnormalities, polysyndactyly.Genetic analysis revealed homozygote mutation (p.Arg154Ter) in *KIF7* gene. *Case6*:3 month-old patient had hypotonia,breathing abnormalities,broad hallux and bilateral syndactyly of toes. *Case 7*:15 year-old patient had broad hallux,partial syndactyly of toes.Genetic analysis of case 6 and 7 revealed homozygous mutation (p.Arg973Ter) in *KIF7* gene.

Results: There are more then 20 genes in JS. Here we suggested two new genes for JS, *PDPR, IFT80*. *PDPR* has not been associated with any disease;It is ought to be

responsible for cleft palate and molar tooth sign and possibly presents a new JS fenotype (Alazami et al.,2017). *IFT80* has been previously described in ATD-2 and we describe its correlation with JS firstly.*KIF7* seems to be the third new gene of JS with a possible founder effect in Northern Turkey.

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P09.075C

Children with CAG expansion in the mild repeat range of Huntingtin gene showing psychiatric but not neurological presentation: is it one more shade of the disease?

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Normal 0 14 false false false IT JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"-Tabella normale"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin:0in; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria; mso-ascii-font-family: Cambria; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Cambria; mso-hansi-theme-font:minor-latin;} Our objective is to characterize the rare occurrence of clinical manifestations in children carrying mutations in the low-mild size, generally causing adult Huntington disease (HD). We are following up a subgroup of young subjects with HD mutation who manifested with disabling psychiatric condition since infancy or adolescence. Among 60 juvenile Huntington disease (JHD) patients we currently follow-up, four of them with mild mutation size showed neurological signs or movement disorders suggestive of HD in adulthood. All patients were genetically (e.g. CAG size analysis) and clinically (e.g. total motor score within the Unified HD Rating Scale) characterized. All four subjects presented a CAG expansion size <45 repeats. Two patients manifested a schizophrenia-like disturbance during the adolescence, with the later appearance of motor signs after age 20. In the other two cases, patients presented symptoms of autistic spectrum disorder, since infancy. One of them showed also a schizophrenia-like disturbance and, later, HD onset with motor signs after 20. One 4-years old patient is currently manifesting an autistic disorder in absence of others neurological signs. The description of JHD is sometime including children with psychiatric manifestations associated with adult motor onset. We advise to pay careful attention to such rare conditions that might represent either psychiatric conditions erreounously classified as JHD or prodromic adult HD cases. <!--EndFragment-->

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P09.076D

Expanding the phenotypic spectrum in neurological disorders associated with mutations in *KARS* gene (lysyl-tRNA synthetase) by the identification of a novel mutation

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Mutations in genes encoding aaRSs (aminoacyl-tRNA synthetases), an essential enzyme family for protein synthesis, were reported in several neurological disorders. aaRSs can be divided into 3 groups according to the cellular localization of the aminoacylation: cytoplasmic, mitochondrial or both. KARS is one of the 3 aaRSs with a bifunctional role and its cytosolic fraction is a component of a multiple aminoacyl-tRNA synthetase (MARS) complex. Biallelic mutations in the *KARS* gene, encoding the lysyl-tRNA synthetase, were described in peripheral neuropathy, non syndromic hearing loss and more complex phenotypes evocating a mitochondrial disorder.

We performed whole exome sequencing in a patient presenting with severe neurological and neurosensory disorder and her healthy parents and we identified compound heterozygous variants in *KARS*, with one novel

mutation. To demonstrate the pathogenic effect of the two mutations, we studied the expression of both KARS isoforms in patient's skin fibroblasts and used a double hybrid interaction study.

We showed a different expression of both KARS isoforms with a decrease of cytoplasmic KARS and an increase of mitochondrial isoform in the patient's fibroblasts compared to a control. With the double hybrid interaction study we showed a defect of interaction between the two mutant forms of KARS and the p38 protein, a core protein responsible for assembly of the MARS complex, which directly interacts with KARS.

In conclusion, we report a patient carrying two mutations in *KARS* gene, with one novel mutation, and presenting in addition to neurosensory deafness and neurological features previously described, cerebellar ataxia and optic neuropathy.

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P09.077A

Case report on a boy with early-onset ataxia and a novel *de novo* KCND3 variant affecting the S4 segment of the Kv4.3 channel

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Introduction: The boy presented at the age of two years with delayed motor, cognitive and language development, dysarthria and postural as well as gait ataxia. Metabolic workup revealed no abnormalities. Today he is 7 ½ years old, receives supportive therapy and attends a special school.

Methods: Whole exome sequencing (SureSelectXT Human All Exon v6) identified the following novel variant in the KCND3 gene: c.910T>C: p.Ser304Pro; segregation analysis showed a *de novo* status. No other pathogenic variants were identified. The variant was classified as likely pathogenic.

Discussion: *KCND3* encodes Kv4.3, a voltage-gated potassium channel with six transmembrane segments, S1-S6, and two intracellular tails. The above variant affects the S4 segment which functions as a voltage sensor. KCND3 pathogenic variants have been associated with Spinocerebellar Ataxia (SCA19/22) with reported ataxia onset ranging from the age of ten to adulthood. Smets *et al*. 2015 have reported on a patient with a *de novo KCND3* variant affecting the S4 segment who showed developmental delay, epilepsy, oral apraxia and attention deficit hyperactivity and first presented with ataxia symptoms at the age of 3 years.

Conclusion: This case supports the findings of Smets *et al*. that *de novo* variants should be considered in SCA. Both reported cases suggest that *KCND3*-associated ataxia should be taken into consideration even in cases with early childhood manifestation and that variants in the segment S4 of Kv4.3 might result in earlier clinical manifestation of ataxia. To our knowledge this is the earliest reported ataxia onset in *KCND3*-associated ataxia.

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P09.078B

An NGS approach to the genetic diagnosis of hereditary leukodystrophies

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Introduction: Hereditary white matter disorders (WMDs) are a heterogeneous group of disorders affecting myelin in the central nervous system, with abnormalities in myelin formation (hypomyelinating leukodystrophies, HLD) or myelin degeneration (demyelinating leukodystrophies, DLD). Although >100 conditions have been identified, <50% of patients receive a genetic diagnosis because of the heterogeneity and complexity of these disorders. Aim of this study was to employ a comprehensive NGS gene panel to study both childhood-onset (EO) and adult-onset (AO) WMD patients negative for the most common genes.

Materials and Methods: A probe-based customized panel covering 142 WMD disease-genes was used to screen 81 index cases with HLD (17 AO; 19 EO) or DLD (21 AO; 24 EO).

Results: Pathogenic mutations were identified in 24,7% of probands (20/81) and likely pathogenic mutations in other 8 probands (9,9%). Overall, the mutation score was higher in the hypomyelinating forms: 47% (9/19) in EO and 24% (4/17) in AO subjects. Interestingly, in AO-HLD forms, we identified mutations in genes usually associated with the more severe EO-HLD forms or spastic paraplegia. Mutation scores in DLD were 21% (5/24) in EO and only 10% (2/21) in AO. Copy number variation (CNV) analysis allowed the identification of deletions/duplications in 6% (5/81) of probands.

Conclusions: Our approach allowed to identify the genetic cause in ~30% of patients, extending the phenotypic spectrum associated to different genes and establishing novel genotype/phenotype correlations. Notably, the high mutation score in AO-HLD patients reveal a genetic cause also for these neglected forms. (Italian MoH grant to CG)

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P09.079C

Trio whole-genome sequencing for patients with unclassified leukodystrophies

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Background: Leukodystrophies are a genetically diverse group of disorders that have in common the selective involvement of the central nervous system (CNS) white matter. They most commonly present in childhood and usually follow a progressive course, with high morbidity and mortality and limited life span. A genetic diagnosis is key to providing accurate prognostic information and reproductive counselling to families, and appropriate clinical management of the patient. Magnetic resonance imaging (MRI) pattern recognition and exome sequencing currently achieve a diagnosis for 70% of patients. **Aim:** To identify the genetic causes underlying a cohort of patients with unclassified leukodystrophy on MRI or negative whole exome sequencing (WES).

Methods: Trio/Quad whole-genome sequencing (WGS) was performed on 27 patients from 22 families with leukodystrophy and a non-diagnostic MRI pattern or non-diagnostic WES.

Results: A genetic diagnosis was achieved in 11/23 families (47%). Implicated genes included six typically associated with leukodystrophy: *DARS2, NDUFV1, BOLA3, COL4A1, TUBB4A, SLC17A5* and five genes not previously-associated with leukodystrophy: *HMBS, FIG4, STAG2, SCN2A, SCN8A*. The latter group includes genes associated with porphyria, epileptic encephalopathy, peripheral neuropathy and congenital malformation. Trio WGS was effective in identifying *de novo* variants as well as variants that had been missed by exome sequencing due to poor coverage.

Conclusions: Results from this cohort advocate the use of trio WGS for the diagnosis of CNS white matter disease as the genetic aetiologies are diverse and may include *de novo* dominant developmental genes as well as recessive housekeeping, metabolic and mitochondrial genes. NHMRC project grant:1068278.

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A novel mutation in the coiled-coil interaction domain of LAMB1 extends the molecular basis of laminin-related cortical malformation phenotypes

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Introduction: Laminins are major components of the basal laminae. *LAMB1* mutations have been reported in only 3 families with lissencephaly and largely overlapping but

distinct phenotypes. Here we present a case with a novel mutation in LAMB1 gene.

Materials and Methods: The patient, 17 year-old girl from a consanguineous marriage, never developed the ability to walk or speak and had drug-resistant seizures. Her physical examination revealed dysmorphic facial features, scoliosis, ocular abnormalities, increased deep tendon reflexes and generalized weakness. The EEG demonstrated generalized epileptic discharges. Cranial MRI showed bilateral perisylvian polymicrogyria. Whole-exome sequencing (WES) was performed using Illumina exome capture (38 Mb) at MIT BROAD Institute. Data analysis was carried out on the RD-Connect Genome-Phenome Analysis Platform. Standard filtering criteria with MAF<1% and high/moderate VEP were used.

Results: We identified a homozygous missense variant (p.Gly1413Glu) in *LAMB1*, associated with lissencephaly 5 (OMIM# 615191). This variant is predicted to be highly pathogenic (CADD=34) and is extremely rare in the control population (0.000824%). The affected residue is localized to the 'Domain alpha', which is between Domain-I and Domain-II that mediate the interaction of laminin chains to form the coiled-coil structure.

Conclusion: Previously reported mutations of *LAMB1* are localized to the EGFLAM and Laminin-IV domains. Our findings for the first time show that a missense mutation in the 'Domain alpha' of LAMB1 can cause a similar phenotype. Moreover, heterogeneity in the clinical phenotype of patients with LAMB1 mutations is an interesting finding that will require functional studies.

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Genetic investigation of the *LIS1*, *DCX* and *TUBA1A* genes in patients with lissencephaly

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Introduction: Lissencephaly is a rare brain malformation caused by abnormal neuronal migration. The main clinical symptoms of the condition are developmental delay, intellectual disability and seizures. Several genes have been implicated in lissencephaly, the most frequently affected are *LIS1 (PAFAH1B1)*, *DCX* and *TUBA1A*. The encoded proteins play an essential role in the formation or function of microtubules.

Materials and Methods: We studied Hungarian patients with isolated (n = 13) and syndromic (n = 2) lissencephaly. Diagnosis was based on clinical evaluation and magnetic resonance imaging. Genetic testing involved sequencing of the *LIS1*, *DCX* and *TUBA1A* genes in the isolated cases and fluorescence in situ hybridization (FISH) for the detection of 17p13.3 microdeletion in patients with Miller-Dieker syndrome.

Results: Genetic analysis revealed pathogenic alterations in 8 patients. We identified three frameshift, one missense, one extension and one nonsense mutations. Three of the intragenic mutations were novel. The *LIS1* gene was affected in 4 patients characterized by agyria/pachygyria. A *DCX* mutation was found in subcortical band heterotopia and the identified single *TUBA1A* mutation was associated with cerebellar hypoplasia and agenesis of the corpus callosum. FISH analysis detected 17p13.3 microdeletion in 2 patients which confirmed Miller-Dieker syndrome.

Conclusions: These are the first results of genetic testing for lissencephaly in Hungarian population. We confirmed the clinical diagnosis in more, than half of the patients. The precise description of the pattern of gyral malformation and associated abnormalities may predict the most likely causative gene in a patient with lissencephaly.

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P09.082B

Integrated analysis of genetic and epigenetic data of healthy individuals with different risk factors for affective disorders

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Affective disorders (major depressive disorder, bipolar disorder) are genetically complex and heterogeneous disorders. Both genetic and environmental risk factors contribute to the etiology of the diseases. However, the neurobiological correlates by which these risk factors influence disease development are hardly understood. Increasing evidence suggests that epigenetic modifications such as DNA methylation have important implications on the development of psychiatric diseases including affective disorders. Several studies revealed that genetic variants can alter DNA methylation at specific loci (methylation quantitative trait loci, meQTLs). To investigate this, we examined the association between genetic variants and methylation levels in whole blood of 44 individuals with different risk factors for affective disorders (genetic/environmental risk). Genotyping of the 44 individuals was conducted using the Illumina Infinium PsychArray. Imputation of the genotypes was performed using IMPUTE2 and 1000 Genomes phase 3 reference haplotypes. DNA methylation was assessed using the Infinium MethylationEPIC BeadChip spanning 850,000 CpG sites. FastQTL using a cis-window size of ± 500 MB and a linear regression model was applied to identify associations between imputed genotypes and methylation levels. In total, we investigated 551,275 SNP-CpG pairs and identified 1,460 significant cis-meQTLs for an FDR of 5% (p-value < $9.06x10^{-8}$). One of the top meQTLs rs4880352 (p-value < $1.72x10^{-10}$) was associated with the methylation level at cg01458105 located nearby *STK32C*. This gene was differentially methylated in a former study of depression in monozygotic discordant twins (Dempster et al., 2014). The meQTLs identified in the present study might improve the interpretation of the functional relevance of genetic variants associated with affective disorders.

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P09.083C

Two patients *with PNKP* mutations presenting microcephaly, seizure, and oculomotor apraxia

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Microcephaly with early-onset, intractable seizures and developmental delay (MCSZ, OMIM #613402) is an autosomal recessive group of heterogeneous disorders, which can also be associated with other severe neurological defects. Mutations in polynucleotide kinase 3'-phosphatase (*PNKP)* have been suggested to cause MCSZ. Additionally, *PNKP* mutations also cause ataxia-oculomotor apraxia type 4 (AOA4) without symptoms of epilepsy or microcephaly. The reported AOA4 cases carried mutations in the kinase domain of *PNKP*. Here we identified three new mutations in two Japanese MCSZ patients who gradually exhibited AOA4 symptoms. All mutations resided within the kinase domain and both patients showed severe epilepsy and microcephaly with congenital anomalies. A 38-year old MCSZ patient showed apparent AOA symptoms with spinocerebellar degeneration. Another patient with MCSZ showed lissencephaly and frequent horizontal headshaking from age 1, suggestive of oculomotor apraxia. Kinase domain mutations in *PNKP* may manifest as a wide spectrum of overlapping phenotypes of MCSZ and AOA4. Therefore, we suggest that each MCSZ and AOA may be a sequential phenotypes of *PNKP* mutations in kinase domain.

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Recessive mutations in *VARS* encoding cytoplasmic valyl tRNAsynthetase cause microcephaly, seizures and progressive cerebral atrophy

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Introduction: Mutations in genes involved in human transcriptional and translational machinery, including the amino acyl-tRNA synthetases (aARSs) family of genes largely account for postnatal neurodegenerative diseases. Herein we investigated the genetic etiology of two siblings with severe early onset neurological manifestations.

Materials and Methods:Whole exome sequencing (WES), homology modeling, RT-PCR, Immunoblotting, *Vars*$^{-/-}$ mouse cell generation and valyl tRNA synthetase (ValRS) enzyme assay were employed during the course of this study.

Results: WES identified novel compound heterozygous mutations in *VARS*, encoding ValRS, one of the aARSs; a missense (c.3192G>A;p.Met1064Ile) and splice site mutation (c.1577-2A>G), that segregated with the affected status. cDNA analysis revealed that the splice site mutation led to nonsense mediated decay, thus resulting in a null allele. Three-dimensional modeling of ValRS predicts that missense mutation lies in the highly-conserved region and could alter side chain packing, thus affecting tRNA binding or destabilizing the interface between the catalytic and tRNA binding domains. Further quantitation of *VARS* expression showed remarkably reduced level of mRNA and protein in patient cells. Aminoacylation experiments revealed markedly reduced enzyme activity of ValRS suggesting the mutations to be loss of function.

Conclusions:Bi-allelic mutations in aARSs are well known for their role in neurodegenerative disorders, yet human disorders associated with *VARS* mutations haven't yet been clinically well characterized. Our study describes the phenotype associated with recessive *VARS* mutations and further functional delineation of the novel mutations that widens the clinical and genetic spectra of individuals with progressive microcephaly.

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P09.085A

Clinical and molecular study of Tunisian families with autosomal recessive primary microcephaly

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Introduction: Autosomal recessive microcephaly or MicroCephaly Primary Hereditary (MCPH) is a genetic heterogeneous disorder, characterized by a reduction in brain volume, with an occipitofrontal circumference (OFC) at birth equal to or less than -2 SD below the mean for sex, age, and ethnicity. An MCPH phenotype has been associated with mutations in at least 18 loci, MCPH1-18. Among them, ASPM (MCPH5 locus) is the most frequently mutated gene reported (60%). In Tunisia, there are no data on genetic variations in this entity.

Materials and Methods: We report the clinical and genetic study of 15 patients belonging to 12 unrelated families presenting congenital microcephaly (OFC between -2 and - 6 SD), an intellectual disability of variable severity, epileptic seizures in 9 patients, and abnormalities on cerebral MRI in 6 patients. We performed 2 multigene panel analyses using next generation sequencing of multiple MCPH-causing genes.

Results: Only two different heterozygous mutations were found in two patients respectively in LIG4 and STIL genes. These variants are not the only causative mutations explaining the observed clinical features. We suggest that other mutations would be present in the regulatory regions of the explored genes, in the non coding regions, or in other genes that were not present in the used panels. WES is planned for these families.

Conclusions: MCPH is a very heterogeneous disorder. Many MCPH families have not yet been ascribed to any of

the known genes, suggesting that additional MCPH genes are still to be discovered.

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Clinical and molecular genetic analysis of a case of familial multiple sclerosis in the Republic of Bashkortostan

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Multiple sclerosis (MS) is a complex disease, and genetic predisposition plays an important role in its development. Familial cases comprise 2% to 5% of all MS patients. In the Republic of Bashkortostan, located in the Volga-Ural region of Russian Federation (RF), 1145 patients with MS have been documented in MS Register; familial cases accounted for 6.1%. Our study focused on a rare case of familial MS - a family of Russian ethnic origin from the Republic of Bashkortostan (RF) that included six individuals with MS in four generations. Pedigree analysis and genotyping of the MS candidate loci were performed. Clinical features of MS in the studied family reflected the most common patterns - matrilineal transmission of the disease, earlier debut and benign course of MS in the younger generations. We observed obvious clinical polymorphism of the disease, in particular, various symptoms of MS debut in affected family members. The absence of clinical exacerbations and lack of active MS foci according to neuroimaging in proband was probably due to the timely administration of immunomodulatory therapy. We found the accumulation in the family of the alleles that were associated with autoimmune diseases according to the results of genome-wide association studies: *ASAP2* rs1109670*C, *GPC5* rs9523762*G, *IL7R* rs10624573*D and rs1494558*I, *STAT3* rs2293152*G, *IL2RA* rs1570538*T and rs12722580*I, *IL2* rs2069772*A. Our results corroborate the hypothesis that several strong-effect genetic variants may be responsible for familial aggregation of MS cases. The study was supported by the RFBR grant No. 17-44-020735.

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P09.089A

Hemochromatosis gene polymorphisms in multiple sclerosis: a meta-analysis

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Introduction: Increasing bodies of evidence support a potential role of iron metabolism in multiple sclerosis (MS). Previous studies examining the association of hemochromatosis (HFE) gene polymorphisms and susceptibility to MS yield inconsistent results.

Materials and Methods: We performed a meta-analysis of six studies conducted in populations of Caucasian origin (1871 patients and 2030 controls) using the Comprehensive Meta-analysis 3.0 software. The strength of association between the HFE C282Y and H63D polymorphisms and MS risk was estimated by odds ratios with 95% confidence intervals. Cochran's Q-statistic and I-squared tests were applied to quantify heterogeneity among studies. Egger's test was used to estimate the publication bias.

Results: The results demonstrated that the HFE C282Y and H63D polymorphisms had no statistically significant association with an increased MS risk (all $P > 0.05$) under subsequent genetic comparison models: dominant model (YY+CY *vs*. CC or DD+HD *vs*. HH) and allelic contrast (Y *vs*. C or D *vs*. H). No evident publication bias or significant heterogeneity among studies was detected.

Conclusions: The present study indicates that the HFE C282Y and H63D polymorphisms are not associated with susceptibility to MS in populations of Caucasian origin. Further studies should be conducted to estimate the contribution of HFE polymorphisms to the progression of MS. National research grants: Genetic analysis of Multiple Sclerosis 13.06.1.1.10 and The role of iron in pathogenesis of multiple sclerosis 13.06.2.2.61

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P09.090B

***In vitro* Investigation for the role of IGF-I and MGF in High Glucose Environment on Neural Stem Cell Proliferation**

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Neural stem cells (NSC) generate neurons, astrocytes, and oligodendrocytes of the nervous system. Mechano-Growth Factor (MGF) is a splice variant of Insulin-like Growth Factor–I (IGF-I), known as a tissue repair factor in different tissues and expressed in brain and heart during ischemic conditions. High glucose levels are harmful to cells. This study aims to determine the intrinsic and extrinsic effects of IGF-I and MGF on NSCs and to observe their proliferative and neuroprotective ability with varying glucose concentrations.

Rat NSC cell-line was applied. Cells were subjected to different glucose levels including 17.5mM: normoglycemia, 27.75mM:diabetes mellitus, 41.75mM:diabetic ketoacidosis and 83.75mM:hyperglycemia-hyperosmolar-state with/without IGF-I ± MGF. NSC proliferation was determined by flow cytometric analysis of Bromodeoxyuridine, and expressions were detected by Real-Time-RT-PCR analysis.

High glucose levels were inhibited NSC proliferation (85,16%, 57,51% and 35,64%, respectively). IGF-I ± MGF were enhanced cell proliferation and re-acquisition of NSC proliferation ($p \leq 0.0005$). There was a negative correlation between IGF-I ± MGF expression and glucose levels. The highest expression with normoglycemia and a dramatic decrease (100 fold) with hyperglycemia were detected. Even at the level of hyperglycemia, increased expressions of IGF-I and MGF (0.5 and 3 fold) compared to the controls were determined.

IGF-I and MGF influence NSC proliferation and increase even at the high glucose concentrations. This study enlightens the IGF-I and MGF role in neuroprotective and neuroproliferative ability of NSC and also for their possible applications in treatment for the patients having diabetic complications.

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Novel phenotype associated with mutations in the PLA2G6 gene

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Novel phenotype associated with mutations in the PLA2G6 gene.

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Pontocerebellar hypoplasia is a group of neurodegenerative disorders. There are 10 known subtyped (PCH1-10) with common characteristics of pontine and cerebellar hypoplasia and atrophy, neocortical atrophy, ventriculomegaly and microcephaly. To date, recessive mutations have been noted in PCH1 in the EXOSC3 gene, in the tRNA splicing endonuclease homolog 54 (TSEN54), mitochondrial arginyl-transfer RNA synthetase (RARS2), and in the vaccinia-related kinase 1 (VRK1) gene.

We present the cases of 2 siblings from a consanguineous Moslem Arabic family with unique combination of progressive cerebellar atrophy and SMA-like anterior horn cell degeneration due to homozygous mutation in the PLA2G6 gene in both siblings.

The *PLA2G6* gene encodes phospholipase A2 beta, which is involved in the remodelling of membrane phospholipids, signal transduction and calcium signalling, cell proliferation and apoptosis.

Mutations in the PLA2G6 are known to cause three main clinical syndromes: infantile neuroaxonal dystrophy (INAD), atypical neuroaxonal dystrophy of childhood-onset (atypical NAD) and adult-onset PLA2G6-related dystonia- parkinsonism .

Our cases have some similarities with INAD. Both syndromes are characterized by rapidly progressive psychomotor regression and cerebellar atrophy. INAD does not exhibit anterior horn cell degeneration.

Though our cases have some similarities with INAD, SMA-like phenotype has never been described in patients with PLA2G6 mutations.

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P09.092D

Functional analysis of a splicing region variant in *C19orf12* in neurodegeneration with brain iron accumulation 4 (NBIA 4)

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Introduction: NBIA4 is an autosomal recessive disorder, caused by homozygous or compound heterozygous pathogenic variants in *C19orf12* gene and characterized by impaired gait, Parkinsonism, behavior and psychiatric symptoms, optic nerve atrophy. We present a case report of an 11-y.o. girl with signs of neurodegeneration with brain MRI typical for iron accumulation.

Materials and methods: Whole exome sequencing (WES) was performed in "Genomed" laboratory, Moscow, Russia. Identified variants were confirmed by Sanger sequencing. DNA was isolated from whole blood using the phenol-chloroform extraction. HEK293T cells were transfected with a minigene plasmid vector containing the splicing region variant. Splicing change was validated by RT-PCR with further Sanger sequencing.

Results: WES identified two variants in the *19orf12* gene, a common pathogenic deletion c.204_214del11 and a novel splicing region variant c.193+5G>A in the intron 2. Functional analysis in HEK293T cells using a minigene plasmid vector showed that c.193+5G>A variant leads to skipping of the exon 2. c.193+5G>A variant disrupts the splicing donor site of the intron 2. This leads to the *C19orf12* exon 2 skipping and results in a frame shift and a formation of the premature stop codon. Hence, a truncated protein is formed which length is less than 25% from the natural.

Conclusion: Therefore, we classify c.193+5G>A variant in the *19orf12* gene as pathogenic and disease-causing in our patient. To our knowledge, this is the first reported pathogenic splicing region variant in NBIA4.

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P09.095C

Identification of *CLN6* and *CLN3* genes mutations in three unrelated Japanese neuronal ceroid lipofuscinosis patients

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The neuronal lipofuscinoses (NCL) are a family of inherited, neurodegenerative disorders that are accumulated with ceroid lipofuscins in neurons, leading to the progressive loss of vision and neuronal impairment. The NCLs have been categorized into four classes based on the age of onset. Kufs disease is an adult onset NCL, which is autosomal recessive progressive lysosomal disorders and responsible genes are *CLN6* and *CLN13*. Here we present the mutational report for adult NCL patients. We have reported a Kufs patient, whose parents were consanguineous marriage (Sakajiri K et al. Intern Med, 1995). We performed gene analysis and found a known but homozygous mutation (c231C>G, pN77K) in CLN6 gene. The amino acid is perfectly conserved among species. In silico analysis, the mutation is predicted to be probably damaging. Moreover, gene analysis for unrelated second Kufs patient was performed, who had a same homozygous mutation. These data suggest that the mutation must be pathogenic one. As they lived in the same district, they seemed to be distant relative or might be inherited founder effect mutation. Gene analysis of the third NCL patient (Ueda T et al. Intern Med, 2013) was performed of CLN6 and 13, however failed to detect any gene mutation. Therefore, we performed whole exome sequencing (WES). We identified a novel heterozygous CLN3 mutation (c313A>G, p. I105V) and autophagy related gene mutation. Since NCLs are very rare disease in Japan and awareness of the adult form of NCL is insufficient, WES will be useful to find causative genes for NCL.

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P09.096D

Identification of a Novel Silent Exonic Point Mutation in the *NF1* Gene Causing Partial Exon 9 Skipping

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Introduction: Neurofibromatosis 1 is a genetic disorder caused by heterozygous mutations in the *NF1* gene. The main features of neurofibromatosis 1 are neurofibromas, café-au-lait macules, axillary or inguinal freckling, lisch noduli, and optic gliomas. We report the identification of a novel silent exonic mutation, which causes partial exon skipping and co–segregate with the disease.

Materials and Methods: We report a family with four family members that fulfils the diagnostic criteria for neurofibromatosis 1. All exons and intron-exon boundaries of the *NF1*, *NF2*, and *SPRED1* genes were sequenced at Department of Molecular Medicine, Aarhus University Hospital by massive parallel sequencing on DNA from the index patient. Carrier testing was performed on three family members by direct sequencing analysis of exon 9. Total RNA extraction was performed from total blood of the index patient using PAXgene Blood RNA Kit 50 v2, and reverse transcribed to cDNA using random hexames. Direct cDNA sequencing was performed using a forward primer

spanning exon 7 and 8 of the *NF1* gene and a reverse primer spanning exon 10 and 11.

Conclusion: The heterozygous c.987A>G mutation in the *NF1* gene was detected in the index patient and two other family members with neurofibromatosis 1 and not identified in a healthy family member. cDNA analysis of the index patient showed, that the mutation causes a partial skipping of exon 9, specifically the last 75bp. We speculate that this silent mutation creates a cryptic donor splice site within exon 9 leading to the production of truncated dysfunctional protein.

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P09.097A

Use of clinical exome analysis in rare neurodegenerative disorders in Serbian population: Firs experience

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Introduction: Neurodegenerative diseases encompass a heterogeneous group of disorders. The clinical diagnosis of neurodegenerative disorders based on phenotype is difficult in conditions with overlapping symptoms. Most of these diseases have a genetic basis and thus are expected to be amenable to genetic or genomic analysis by next-generation sequencing (NGS).

Material and Methods: Study included 20 patients with various neurodegenerative diseases, negative after standard molecular-genetic testing. Preference was given to family cases with early presentation or complex phenotype suggesting genetic heterogeneity. Clinical exome sequencing (CES) was performed using TruSight One Panel on Illumina MiSeq NGS platform. Variants in genes related to neurodegenerative diseases were analysed using Illumina Variant Studio v3; confirmation by Sanger sequencing was done.

Results: We revealed 10 pathogenic or likely pathogenic variants in 9 different genes related to rare neurodegenerative disorders in 9 patients. Mutations in *PANK2*, *PDGFB*, *DCTN1* and *PSEN1* genes causing neurodegeneration with brain iron accumulation, Fahr's, Perry syndrome and Alzheimer's disease respectively, are compatible with the phenotype of patients. In five cases DNA diagnosis remain unclear. Only one recessive mutation was found in *SPG7* and *AP5Z1* gene in cases of spastic paraplegia, and in *COL6A3* gene in case of dystonia. In another two cases detected variants were not directly related to patient's phenotype (*HSPB3* mutation in Amyotrophic lateral sclerosis, and *MYH14* mutation in patient mitochondrial myopathy).

Conclusion: These results signify the importance of CES in better diagnosis of obscure cases with neurodegenerative disorders and gave us better insight in complexity of genetics of these disorders.

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P09.098B

Biallelic mutations in the homeodomain of NKX6-2 underlie a severe hypomyelinating leukodystrophy

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Introduction: Hypomyelinating leukodystrophies are genetically heterogeneous disorders with overlapping clinical and neuroimaging features reflecting variable abnormalities in myelin formation. The homeobox protein NKX6-2 is a transcription factor regulating multiple developmental processes with a main role in oligodendrocyte differentiation and regulation of myelin-specific gene expression.

Materials and Methods: Whole-exome sequencing (WES) and homozygosity mapping of selected patients from three unrelated families was undertaken. The variants identified were validated by Sanger sequencing and cosegregation analysis.

Results: Five affected subjects (three unrelated families) were documented to share biallelic inactivating mutations affecting the NKX6-2 homeobox domain. A trio-based whole exome sequencing analysis in the first family detected a homozygous frameshift change [c.606delinsTA; p.(Lys202Asnfs*?)]. In the second family, homozygosity mapping coupled to whole exome sequencing identified a homozygous nucleotide substitution (c.565G>T) introducing a premature stop codon (p.Glu189*). In the third family, whole exome sequencing established compound heterozygosity for a non-conservative missense change

affecting a key residue participating in DNA binding (c.599G>A; p.Arg200Gln) and a nonsense substitution (c.589C>T; p.Gln197*), in both affected siblings. The clinical presentation was homogeneous, with four subjects having severe motor delays, nystagmus and absent head control, and one individual showing gross motor delay at the age of 6 months. All exhibited neuroimaging that was consistent with hypomyelination.

Conclusion: The finding of individuals with a severe neurodevelopemental phenotype with hypomyelination associated with biallelic mutations in *NKX6-2* provides direct evidence of the relevant role of NKX6-2 in CNS development in humans.

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P09.099C

NOTCH3 mutations in Serbian CADASIL patients

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Introduction: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is one of the most common inherited small-vessel disease, presenting with recurrent subcortical stroke episodes, migraine, mood disorders and dementia. CADASIL is caused by *NOTCH3* gene mutations, and majority of confirmed pathogenic variants are alterating number of cysteine residues in EGFr domains of the NOTCH3 protein. CADASIL patients usually have positive family history and *de novo* mutations are not commonly reported.

Materials and Methods: Total genomic DNA was extracted from peripheral blood leukocytes of 249 Serbian patients with clinical diagnosis of CADASIL (with or without family members with similar symptoms) and family members of patients with confirmed mutations. PCR amplification and direct sequencing of exons 2-6 of *NOTCH3* gene were performed.

Results: Nineteen heterozygous mutation carriers were identified in our cohort and mutation frequency was 7,6% (19/249). Eight previously described, cysteine-alterating mutations were identified in 13 cases. Two changes (Cys67Gly and Gly89Cys) were first described in Serbian CADASIL patients and characterized as pathogenic according to *in silico* prediction. Positive family history was reported in 10 of 19 mutation carriers. Interestingly, parents of one mutation carrier were tested and found negative for *NOTCH3* mutation.

Conclusions: Two novel mutations and one confirmed *de novo* mutation in our study are implicating that estimation of *NOTCH3* gene mutation frequency and type in different populations, as well as consideration of possible *de novo* mutations, is important for efficient genetic testing strategy for CADASIL.

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P09.100D

A novel case of severe neurodevelopmental delay in a patient with Pitt-Hopkins like 2 syndrome associated with compound heterozygous deletion in NRXN1

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Introduction: Neurexin 1 is a key organizer of the synapse. Only nine cases with compound heterozygous NRXN1 deletions/mutations have been reported so far, all of which share a Pitt-Hopkins like 2 syndrome phenotype, characterized by a severe global developmental delay. We describe a 5 y.o. child with a compound heterozygous deletion in NRXN1 gene and typical phenotype.

Materials and Methods: The patient was evaluated by GMDS and VABS Scales. CGH-array (180 K, Agilent Technologies) was performed on the patient and the NRXN1 deletions and familial segregation were confirmed by qPCR .

Results: the patient shows a severe global developmental delay with intellectual disability, poor speech, motor stereotypies, autistic features and cranio-facial dysmorphism. In addition, the child suffers from celiac disease with chronic constipation and positive anti-gliadin IgG. Behavior, as assessed by GDMS, is equivalent to an age of <12 months. Array-CGH revealed a complex alteration of the region 2p16.3 due to the presence of two inherited,

partly overlapping, 467 and 269 kb deletions, both disrupting the NRXN1 gene.

Conclusions: We found significant overlap in phenotypic severity between our case and the nine previously reported patients with biallelic defects in NRXN1. Our report confirms the evidence that NRXN1 nullisomy is associated with the clinical diagnosis of Pitt-Hopkins like 2 syndrome.

Carriers of an heterozygous NRXN1 deletion/mutation showing a particularly severe phenotype should be screened for possible mutations or microdeletions of the second allele.

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P09.101A
Phenotypic characterization of olfactory reference syndrome in patients presenting to genetics for query trimethylaminuria

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Olfactory reference syndrome (ORS) is a psychiatric disorder in which individuals hold a false belief that they emit an offensive body odour. ORS leads to significant distress, and yet remains poorly characterized with limited recognition and characterization of its management. Patients with ORS rarely seek psychiatric attention as first-line treatment, and instead seek an organic diagnosis, such as trimethylaminuria (TMAU). TMAU is an inherited disorder in which there is a failure to break down trimethylamine, creating a pungent fishy odour. This study characterizes the clinical and demographic features of a cohort who meet the definition of ORS, and yet who presented to a Canadian genetics clinic for query TMAU. Data was obtained via a retrospective chart review over a 7 year span (N=54). Only two individuals who presented for query TMAU had this diagnosis, while 83% had a likely diagnosis of ORS. Of this ORS group, 62% were female, 73% had a psychiatric history, and 77% were seen by multiple specialties. This study is the first to systematically examine a large, ethnically diverse group of patients who fit the definition of ORS. Based on this phenotypic characterization, we suggest clinical criteria to assist in the recognition of ORS. Improving the recognition and diagnosis of ORS will lead to more targeted management, reduce psychological distress and stigma, and save in health care costs. The unique phenotypic features also suggest a possible as-yet undefined genetic basis for this syndrome, which future genomic studies could help to elucidate.

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P09.104D
Identification of a new candidate gene of monogenic Parkinkison disease: MTIF3

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Parkinson disease (PD) is a chronic, debilitating and progressive neurodegenerative disorder characterized by bradykinesia, resting tremor, rigidity and postural instability.

About 5-10% of patients suffer from a monogenic form of PD caused by highly penetrant mutations. Currently, sixteen PARK loci have been identified with autosomal dominant or autosomal recessive genes.

We report here a 48 years old patient with PD. Resting tremor was initially limited to the left upper limb. As it is commonly observed in early onset PD patients (Mehanna), symptoms other than tremor were more evident at the beginning of the disease with decreased arm swing movements, depression, bradykinesia and urinary incontinence.

Once discarded the presence of pathogenic mutations within the known genes causing PD. The analysis of whole exome data applying a prioritization scheme identified two pathogenic variants which encode premature stop codons in MTIF3 gene that interacts with PINK1, a known PD autosomal recessive causing gene.

Mitochondrial damage plays a central role in PD pathogenesis and several PD causing genes are known to regulate mitochondrial function and homeostasis Moreover, a known allele of the mitochondrial factor 3 (MTIF3) which causes a significant reduction of MTIF3 mRNA expression, has been associated to PD in patient-control series.

If paucity of MTIF3 increases the risk of PD, Its complete absence should be related to significantly higher risk.

Our data suggest that MTIF3 could be a new PD causing gene. Further functional and population studies are needed to assess the exact role of MTIF3 in the pathogenesis of PD.

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P09.105A

Identification of new genes involved in autosomal dominant forms of Parkinson's disease

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Parkinson disease (PD) is the second most frequent neurodegenerative disorder, affecting 1% of the population above 65 years. To date, the identified genes associated with AD PD only explain 10-15%, so many genes remain to be discovered. We selected 73 affected relatives from 20 multiplex AD PD families for exome sequencing. We looked for rare heterozygous variants, predicted to be pathogenic using CADD and M-CAP scores, shared by all affected relatives within the family. Replication studies were done using the available exome data from ~1,500 PD cases and 560 controls from the IPDGC consortium. Pathway analyses were realized using the R package Clusterprofiler and the web interface ConsensuspathDB. For each family, we obtain a list of candidate genes (2 to 30). To prioritize them, we applied filtering criteria which in order were 1) expression in brain; 2) replication of these possible candidate genes in additional families; 3) sharing common physiopathological pathways. None of the identified candidate genes was replicated, using home and IPDGC consortium exome databases. On the other hand, using Clusterprofiler we found that some of these genes can be grouped in the same biological process, which is actin metabolism. Furthermore, using ConsensuspathDB we highlighted that some of these candidates' genes were able to interact with genes involved in PD. *Conclusion* Although we identified a substantial number of multiplex families with AD PD, none of these families was explained by possible shared candidate genes, highlighting the genetic heterogeneity of PD. However, these genes were enriched in a common regulating.

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P09.106B

Targeted genetic analysis of Parkinson disease in Bulgarian patients

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Parkinson disease (PD) is the second most common neurodegenerative disease resulting from the interplay of multiple genes with environmental risk factors. Inheritance is in an autosomal dominant, autosomal recessive, or X-linked manner and monogenic forms are rarely found. The main goal of the current study is to evaluate the frequency and type of mutations in genes associated with Parkinson disease in Bulgarian patients. Altogether, 69 patients with PD and detailed clinical assessment and 108 healthy controls, matched by age, gender and ethnicity (NC) were recruited. All individuals were analyzed with a custom panel including known and candidate PD genes on an Illumina NGS platform. All pathogenic variants were confirmed with Sanger sequencing. 14 patients were analyzed with MLPA P051-D1 Parkinson kit. In 52% (36/69) of the patients variants in *LRRK2, PARK2, PINK1, PARK7, ATP13A2, FBXO7, PSEN1, PSEN2, CHMP2B, GRN, MAPT, EIF4G1* were identified. Altogether 14 new variants were found (10 missense, 2 splice site, 2 frameshift) and 14 VUS (13 missense, 1 splice site). Four pathogenic variants, including two novel ones, were identified in *PARK2, PARK7, PSEN2*. No mutations were found with MLPA analysis. This is the first Bulgarian study including a cohort of 69 patients showing the frequency and distribution of novel and known variants in genes associated with Parkinson disease. Our findings contribute to a better understanding of the molecular basis of Parkinson disease and have implications for diagnostic testing and genetic counseling in Bulgarian population. Acknowledgements: D35/27.05.2016, D77/02.05.2017 of SF, MU-Sofia, DUNK01-2/2009, NSF.

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P09.107C

Bridging mitochondrial and lysosomal dysfunction in Parkinson Disease: clues of oligogenic inheritance

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Introduction: Mutations in *PARK2*, *PINK1*, *DJ-1* and *VPS13C* cause autosomal recessive Parkinson Disease (PD) and impair mitochondrial quality control pathways. Moreover, substantial evidence highlights the importance of lysosomal mechanisms in PD, including excessive burden of lysosomal storage disorder (LSD) gene variants in PD patients.

Materials and Methods: We analyzed WES data of 66 PD patients, including 34 patients with a single rare heterozygous mutation in an autosomal recessive PD gene and 2 related patients, to investigate a role for different genetic factors in disease etiology. Variants in autosomal recessive genes associated with PD, atypical parkinsonian syndromes and LSD were prioritized based on quality, frequency in public databases and impact (splice site and non-synonymous variants with Combined Annotation Dependent Depletion score >20).

Results: The WES data analysis revealed the presence of oligogenic inheritance through known pathogenic and rare novel heterozygous mutations in multiple genes. We identified 1 patient carrier of compound mutations in *PARK2/DJ-1* and 1 patient with compound mutations in *PARK2/VPS13C*. Coexistence of mitochondrial and lysosomal pathways is established by the observation in 16 patients of multiple mutations in PD and LSD genes, including 7 known pathogenic LSD gene mutations. Of note, the compound mutations *PARK2* p.P437L and *HEXA* p.R247W are both present in an affected mother and daughter. Additionally, we found 4 carriers of compound mutations in different LSD genes.

Conclusions: Our results underpin the potential oligogenic complexity of Mendelian genes in PD etiology and highlight the crosstalk between mitochondria and lysosomes in the pathophysiology of PD.

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P09.108D

MosSCI and CRISPR *C. elegans* models of LRRK2 related Parkinson's disease fail to produce the expected phenotype

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Parkinson disease (PD) results from the loss of dopaminergic neurons and represents one of the most common neurodegenerative diseases. Models for LRRK2-mediated PD in *C. elega*ns have relied on multi-copy overexpression of human transgenes from extrachromosomal arrays or random genomic integrations. Although successful in reproducing the neurotoxic effects of human *LRRK2* mutants, the variability of expression between worm strains in these models complicates the interpretation of LRRK2 mutant phenotypes. To produce a closer physiological model of PD in *C. elegans* we generated new strains in which transgenes are integrated in a defined genomic locus in single copy. This ensures that human wild-type *LRRK2* and mutant transgenes are expressed at identical levels in every animal. We also used CRISPR-Cas9-mediated genome editing to introduce the equivalent of the human *LRRK2* G2019S variant into the worm orthologue *lrk-1*. We show that expression of either human mutant *LRRK2* (at similar levels of endogenous *lrk-1*) or the worm *lrk-1* mutant does not lead to a dopaminergic neurodegenerative phenotype or motility problems, both a major hallmark of *C. elegans* PD models, even in severely aged animals. In fact, mutant strains show a minor increase in the thrashing rate along with the *cat-2* mutant. Other lesser behavior deficits were also present, such as reduced life span and hyperactive egg laying. While the newly generated models do address the issue of variable expression neither mutant human *LRRK2* nor mutant *lrk-1* produce a robust easy to score phenotype, and therefore cannot be used for genetic or pharma screens.

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Genomic analysis of coding and regulatory regions using NGS in early-onset parkinsonism: a multicentric study

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Aims: Next-generation sequencing (NGS) has proven to be very useful for the diagnosis of heterogeneous disorders in both their etiology and clinical expression. The aim of this study is to evaluate whether genetic diagnosis of patients with early-onset parkinsonism (EOP) can benefit from a panel that contains both coding and regulatory regions of genes related to these disorders.

Materials and methods: we have recruited 96 EOP patients (<55 years of age) from the Movement Disorders Units at tertiary centers. The custom genetic panel of Nextera® Rapid Capture Enrichment technology (Illumina) contains 63 genes related to the following: i) familiar Parkinson disease, ii) atypical Parkinsonisms, iii) Parkinsonism-pyramidal syndrome and iv) risk factors for Parkinson disease. MiniSeq Sequencing System (Illumina) was used for NGS and bioinformatics analyses and filtering were performed.

Results: Until date, we have sequenced 48 patients. We found 91.2% coverage of targeted genomic regions at 50-fold mean coverage. These results show 71 previously documented rare variants and 19 new variants in 35 out of 63 genes of the panel. 13 rare variants where reported as pathogenic and 19 novel variants had *in silico* prediction of being pathogenic. We also have detected Copy Number Variations (CNVs) in 28 samples. Currently, we are analyzing regulatory regions variants.

Conclusions: Our preliminary findings show that NGS panels can be useful to identify gene variations in EOP patients and it shows novel genotype/phenotype relationships.

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Generation of prediction models based on rare and common genetic variants in PD

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Introduction: Polygenic risk scores (PRS) are widely used in the field of genetics to associate a group of SNPs to a disease/trait. However, majority of the studies use only the common genetic variants to generate the PRS. In our study, in addition to the common genetic variants, we also used the number of private loss of function variants per sample (singleton_score).

Materials and Methods: The data was provided by Parkinson's Progression Markers Initiative consortium. We used the NeuroX array data to generate the PRS based on common genetic variants and Whole exome sequencing data to generate singleton_score. Machine learning models were built based on state of the art methods and we performed a comprehensive assessment of the contribution of singleton_score in the prediction models.

Results: We confirm that the addition of singleton_score significantly improved the performance of the model (AUC=0.72) compared to the model built on common variants alone (AUC=0.62). Additionally, we show that the inclusion of singleton_score along with the clinical scores improved the overall predictive ability of the model.

Conclusions: Although PRS generated by using common variants is a very useful approach in order to understand the disease mechanism. It is interesting to see that rare/ultra-rare variants also contributing to the association signal, providing evidence that rare/ultra-rare might add to missing heritability of PD.

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GBA-related Parkinson disease: mutational frequency and clinical- biochemical correlates in the Italian population

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Introduction: Heterozygous variants in the *GBA* gene, encoding β-glucocerebrosidase (GCase), are major genetic risk factors for Parkinson Disease (PD). Their frequency and phenotypic correlates have been assessed in many large cohorts of different ethnicities, being identified in ~10% PD. However, in the two published Italian studies, only two common alleles (N370S and L444P) were investigated, with an overall frequency of 4,4%.

Patients and Methods: The whole *GBA* coding region (11 exons) was Sanger-sequenced in 850 Italian PD probands, who underwent deep phenotyping for motor and non-motor signs. Clinical features and, in a subset, GCase activity were compared between *GBA*-PD and not mutated-PD (NM-PD), and among carriers of complex, severe, mild and risk variant alleles.

Results: In this large cohort, the frequency of *GBA* heterozygous variants was 14,5%, much higher than previously reported in Italy. 32 distinct variants were identified, of whom N370S and L444P accounted only for 48% *GBA*-PD.

GBA-PD significantly differed from NM-PD for earlier, prevalent bradykinetic onset and higher occurrence of non-motor features, including cognitive, psychiatric and autonomic dysfunctions. A more aggressive course and a higher predisposition to dementia correlated with complex/severe alleles and with risk variants, respectively. GCase activity was significantly reduced in PD-*GBA*, without significant differences among sub-classes.

Comments: *GBA* heterozygous variants are a common risk factor for PD in Italy, and a complete gene screening is warranted to properly identify gene carriers. This is relevant for counselling, appropriate management of non-motor complications, and selection of patients amenable to enter ongoing clinical trials.

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P09.112D

Parkinson's disease: a disruption of nuclear/mitochondrial DNA communication

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Background: *The role of mitochondrial function in Parkinson's disease (PD) is established, however the precise role of mtDNA (mtDNA) variation in PD remained unclear. Work in our laboratory has focused in the role that inherited mtDNA variants play in developing PD, identifying both low- and high-risk mtDNA alleles associated with PD. Based on this, and supported by our work in other diseases, we hypothesised that inherited mtDNA variation disturbs the transcriptomic balance within vulnerable brain regions, causing either a direct mitochondrial phenotype or a disruption of the delicate mitochondrial/nuclear synergy contributing to cellular vulnerability and ultimately leading to PD.*

Methodology: *To address this hypothesis, we interrogated the transcriptome of vulnerable regions of the brain in post-mortem PD tissue using RNAseq, stratifying our results using background mtDNA sequence data and comparing transcript abundances to matched controls.*

Results: *Our results indicate the background mtDNA sequence modulates the expression of key protein coding transcripts. PD cases carrying high-risk alleles showed differential expression of components of the lipid metabolism or vesicular trafficking pathways; supporting our own recent metabolomic observations in PD. Conversely, PD cases carrying low-risk alleles showed differential expression of components of the calcium homeostasis or apoptosis pathways, supporting recent expression and proteomic observations in PD.*

Conclusion: *Our experiments, for the first time, reveal a link between inherited mtDNA variation and the nuclear transcriptome - suggesting that inherited mtDNA variation*

can impact cellular vitality in neuronal tissue; making a significant contribution to the observed mitochondrial dysfunction and neuronal cell death seen in PD.

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Deep mitochondrial sequencing in Parkinson's disease reveals possible genetic modifiers of penetrance in Parkin/PINK1 mutation carriers

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Background: Biallelic mutations in *Parkin* and *PINK1* are fully penetrant and cause recessively inherited Parkinson's disease (PD). On the other hand, heterozygous mutations in these genes may be considered as a risk factor for PD or even act in a dominant manner with highly reduced penetrance. Since both Parkin and PINK1 function in the removal of dysfunctional mitochondria, we hypothesize that mitochondrial DNA (mtDNA) mutations are both a consequence of dysfunction in these genes, and also able to influence age-dependent penetrance and thus the onset of disease symptoms if not cleared sufficiently over time (i.e. a 'second hit').

Method: We performed deep mtDNA sequencing in 124 individuals with Illumina NextSeq in blood-derived DNA to assess mitochondria mutational load including somatic mosaicism and individual mtDNA variants. Patients were recruited from Germany and Italy and comprised carriers of PINK1 ($n = 29$) and Parkin mutations ($n = 52$), idiopathic PD patients ($n = 23$) and controls ($n = 20$).

Results: A mean coverage of >10,000X was achieved with high sensitivity of detecting low level heteroplasmic (<15%) variants. Parkin mutation carriers have more variants including both single nucleotide variants (SNV) and copy number variations (CNV) compared to controls and idiopathic PD ($p = 0.005$). Lastly, comparing early and late onset Parkin mutation carriers, we identified a potential protective variant in *ATP6* (p.A177T).

Conclusion: Parkin/ PINK1 mutations may predispose to an increased mitochondrial mutational load. Replication and random segregation of heteroplasmic mitochondrial DNA, alongside cellular mechanisms to correct mitochondrial health, may partially explain the modified penetrance in (heterozygous) Parkin/PINK1 mutation carriers.

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P09.114B

Genotype-phenotype correlations in an Italian sample of patients with Phelan-McDermid syndrome

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Introduction: Phelan-McDermid syndrome (PMS), is a neurodevelopmental disorder characterized by intellectual disability (ID), hypotonia, delayed or absent speech, and autism spectrum disorder (ASD). PMS is due to de novo chr. 22q13 terminal deletions or point mutations involving the SHANK3 gene, crucial to the formation and plasticity of excitatory synapses. Disruption of SHANK3 causes approximately 0.5% of ASD cases, with higher rates reported in ASD co-morbid with ID. This study aims to assess genotype-phenotype correlations in 42 Italian PMS patients.

Materials and Methods: For each patient, we collected medical history and performed neurological examination, behavioral observation, medical work-up and psychodiagnostic testing (Leiter or Raven, ADOS, ADI-R, VABS, VAS, CGI, CBCL, SCQ, SSP, WHOQOL, QOL-A, ABC and RBS-R). Array-CGH using the Human Genome CGH Microarray 4 x 180K or 400K Kit (Agilent) or targeted Sanger sequencing were performed.

Results: The clinical phenotype of PMS patients displays great interidividual variability. Larger deletions are associated with more severe clinical phenotypes and developmental delays. However, similar deletions result at times in phenotypes differing significantly in severity. Some phenotypic features are highly correlated with a positive family history.

Conclusions: Interindividual differences in the PMS phenotype are sizable and may stem from at least three different sources: (a) deletion size involving other functional genes in addition to SHANK3, (b) additional mutations, microdeletions, or epigenetic influences in the

non-deleted 22q13 allele, (c) greater penetrance of familial genetic loading for neuro-behavioral disturbances in the presence of a SHANK3 synaptopathy.

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P09.115C

Further delineation of the genotype-phenotype correlation associated with PIGC mutations

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Phosphatidylinositol glycan anchor biosynthesis class C (*PIGC*) encodes for an endoplasmic reticulum protein essential for the first step of the biosynthesis of the glycosylphosphatidylinositol (GPI) that anchors more than 150 proteins to the cell surface (GPI-anchored proteins, GPI-APs). These proteins are important for development, neurogenesis, and immunity. Inherited GPI deficiencies (IGDs) cause intellectual disability, epilepsy, coarse facial features, and multiple organ anomalies and are inherited as autosomal recessive traits. Whole exome sequencing identified a homozygous variant in *PIGC* gene (NM_153747.1: c. 859G>T, p. E287*) in a 3-year-old female patient with macrostomia, tented upper lip, low-set ears, drug-resistant epilepsy and severe global developmental delay. At present she has not achieved any developmental milestones. Brain imaging showed diffuse brain atrophy. She was born from healthy parents. This variant is reported in dbSNP 150 as rs770671752 and has a minor allele frequency (MAF) of 1.422e-5 (3/211,000) in gnomAD. No homozygotes have been reported on public databases. *PIGC* pathogenic mutations were recently identified in probands of two unrelated families with epilepsy and intellectual disability. Moreover, our analyses of patient's leukocytes showed that the expression of CD16 (GPI-anchored membrane receptor) and FLAER (marker for all GPI-APs) was significantly decreased providing further proof that *PIGC* variants affect membrane expression of GPI-APs. In conclusion, our family is the third reported with *PIGC* mutations, helping to better delineate the genotype-phenotype correlation and further corroborating the role of this protein in neurodevelopment.

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SLP-2 rescues PINK1 deficiency in a cellular model and in *Drosophila*

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Introduction: Deficiencies of complex I activity have been observed in the *substantia nigra* of Parkinson's disease (PD) patients, and loss of *Parkin* and *PINK1* results in the reduction of complex I activity shown in cell and animal models. The two key PD genes related to mitochondrial function are Parkin (*PARK2*) and PINK1 (*PARK6*). Recently, we have shown that Parkin interacts with mitochondrial Stomatin-like protein 2 (SLP-2), which functions in the assembly of respiratory chain proteins. Induced overexpression of SLP-2 was able to correct for mitochondrial alterations caused by Parkin deficiency. The aim of the present study was to extend this work to test whether SLP-2 is also able to rescue mitochondrial dysfunction induced by PINK1 knockdown in multiple systems.

Materials and Methods: PINK1 deficiency was induced by siRNA-mediated knockdown in SH-SY5Y

neuroblastoma cells and *pink1* RNAi in *Drosophila*. Several mitochondrial phenotypes were assessed.

Results: The PINK1 siRNA cells exhibited significantly increased mitochondrial superoxide levels and decreased mitochondrial membrane potential and complex I activity. Induced overexpression of SLP-2 significantly rescued the identified mitochondrial dysfunction. *In-vivo Drosophila* studies showed a genetic interaction of PINK1 and SLP-2, and further, overexpression of SLP-2 transgenes rescued *pink1* mutant phenotypes, in particular loss of dopaminergic neurons.

Conclusions: These results highlight a protective effect of SLP-2 not only in Parkin-deficient cells but also in PINK1 deficiency, pointing to SLP-2 as a novel molecular target able to boost mitochondrial function and neuronal survival in multiple PD genes that disrupt mitochondria.

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P09.118B

Association between Inflammatory and Metabolic markers and the Polygenic Risk Score of Schizophrenia in First Episode Psychosis

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There is an increasing interest in the clarification of the different components that characterize the genetic vulnerability risk for psychotic disorders. In this work, we analyzed the association between Polygenic Risk Score (PRS) for schizophrenia and the serum levels of 19 different inflammatory/metabolic markers in 33 controls and 83 First-Episode psychosis (FEP) patients. The diagnosis of FEP was schizophrenia (n = 46), bipolar disorder (n = 35) and delusional disorder (n = 2). The PRS was calculated according to the summary association results for schizophrenia available from the Psychiatric Genomic Consortium (PGC). Genome-wide and pathway specific PRS regarding 186 KEGG pathways were computed. The associations between markers serum levels and PRS were evaluated considering p-value and R2 of linear regression analyses. We identified a significant positive association of schizophrenia PRS with the inflammatory marker C-C Motif Chemokine Ligand 4 (CCL4, R2=0.10, adjusted p = 7.86e-3) and a significant negative association with the hormone ghrelin (R2=0.11, adjusted p = 6.84e-3). Noteworthy, in our sample CCL4 levels were increased in patients compared to controls, while ghrelin levels were decreased. Interestingly, pathway specific PRS analysis showed that genes involved in different pivotal metabolic pathways (e.g., related to amino acids and nucleotides synthesis/degradation) are the ones leading the associations for both CCL4 and ghrelin. These results suggest the presence of a potential correlation between the genetic component for schizophrenia and immune/metabolic serum markers levels at the onset of FEP, indicating an involvement of these processes in the psychosis vulnerability.

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Mutations of *KIF14*, encoding a kinesin-3 family member of microtubule motors, cause primary microcephaly

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Autosomal recessive primary microcephaly (MCPH) is a rare condition characterized by a reduced cerebral cortex accompanied with mild to severe intellectual disability. Mutations in 17 different genes have been shown to cause this phenotype. Recently, mutations in *CIT* encoding a component of the central spindle matrix were described in MCPH families as well as in syndromic cases of microcephaly. Here, we report on two MCPH families, one from Pakistan and the other from Saudi Arabia, with three

affected individuals each, in which we identified homozygous mutations in *KIF14* (NM_014875.2;c.263T>A; pLeu88* and c.4071G>A;p.Gln1357=, respectively) as the likely cause. Further, in a German patient presenting with a severe form of primary microcephaly and short stature, we identified compound heterozygous missense mutations in *KIF14* (NM_014875.2;c.2545C>G;p.His849Asp and c.3662G>T;p.Gly1221Val). Interestingly, all but one (p.His849Asp) of the identified mutations impaired splicing and resulted in a truncated protein. Intriguingly, *Kif14* knockout mice also showed primary microcephaly. Human KIF14, localizes at the midbody to finalize cytokinesis by interacting with CRIK (Citron Rho-interacting kinase). We found impaired localization of both KIF14 and CRIK at the midbody in patient-derived fibroblasts. Further, we observed a large number of binucleated and apoptotic cells — signs of failed cytokinesis that we also observed in experimentally KIF14-depleted cells. Thus, in keeping with previous findings on *CIT* mutations, our data underline the role of an impaired cytokinesis for the etiology of primary and syndromic microcephaly.

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Identification of novel mutations in the *PRNP* gene in patients with Creutzfeldt Jacob disease

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Introduction: Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of neurodegenerative disorders that affect 1-2 per million habitants. TSEs are classified into three diseases based on their clinical and neuropathological characteristics: familial Creutzfeldt-Jakob disease, Gerstmann-Straßussler-Scheinker disease, and familial fatal insomnia. According to their presentation they are classified into three groups: a) sporadic of unknown etiology (85%), b) genetics, caused by mutations in the PRNP gene (10-15%), and c) acquired (<1%). The abnormal accumulation in the neurons of the prion protein, leads to apoptosis and cell death, presenting a rapidly progressive dementia with motor characteristics and a short survival time from the beginning (usually 1 year). In the present work the genealogical, clinical and molecular study of four cases of familial Creutzfeldt-Jacob disease is analyzed. Objective: To molecularly describe for unrelated familial cases with Creutzfeldt-Jacob disease.

Materials and Methods: From peripheral blood, the genomic DNA of four patients was obtained by conventional techniques. The coding region of PRNP was amplified and sequenced through PCR and DNA automated sequencing. Results were compared with 100 healthy controls and world databases.

Results: Four heterozygous mutations were identified in the PRNP gene: c.598G>A, c.586G>A, 3 c.586G>A and c.598G>A.

Conclusions: Through the sequencing study, four mutations in the *PRNP* gene were identified in the four patients. With the corresponding ethical considerations, the confirmation of the mutations is useful to analyze later the descendants or siblings of the affected patients, in order to provide an adequate genetic counseling.

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P09.121A

Whole exome sequencing identifies novel etiologies in a cohort of patients with progressive myoclonus epilepsy

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Progressive myoclonus epilepsies (PMEs) comprise a group of clinically and genetically heterogeneous rare disorders manifesting with action myoclonus, tonic-clonic seizures, ataxia and progressive decline with typical onset in childhood. We carried out WES in a cohort of PME patients negative for the minisatellite expansion mutation in *CSTB* and the recurrent *de novo* mutation in *KCNC1* as well as for various PME associated genes.

We studied 41 independent cases including 31 with no family history. To enhance identification of *de novo* mutations a trio approach was used in 24 cases. The overall bioinformatic analysis included 44 additional cases in whom an earlier singleton WES study was unrevealing. Four patients had pathogenic variants in known but rare

PME-associated genes, *ASAH1* and *CERS1*, both involved in the sphingolipid pathway, as well as in *NEU1*, coding for a lysosomal sialidase. Furthermore, identification of novel pathogenic variants in two neurodevelopmental genes previously not associated with PME, *CHD2* and *NAXE*, were confirmed.

In addition, through identification of pathogenic variants in *NUS1* and *DHDDS*, this study extends the pathomechanistic etiology of PMEs to protein glycosylation, with NUS1 directly interacting with DHDDS. Functional assays in fibroblasts of a patient with a *de novo* frameshift alteration in *NUS1* confirmed the underlying protein glycosylation defect. Likely pathogenic variants identified in further genes provide novel insights into the molecular basis of PMEs and imply that the as yet unsolved cases of PME are a highly heterogeneous group of ultra-rare disorders.

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P09.122B

Differential DNA methylation associated with the onset of psychiatric symptoms

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Background: The study of psychiatric symptoms (PS) instead of diagnosis could help to better understand the basis of psychiatric disorders. We aimed to identify differentially methylated positions (DMPs) and regions (DMRs) associated with PS. For that, we compared: 1) children and adolescents with low PS at baseline and with high PS after 3 years of follow-up (categorical comparison); 2) PS as a score (continuous comparison).

Methods: PS were assessed using Child Behavior Checklist (CBCL). For the present study, we selected from a large Brazilian study 24 subjects with CBCL score < 30 at baseline that increased CBCL more than 16 after 3 years of follow-up. We generated methylation data using EPIC BeadChip. In continuous comparison, we used Delta CBCL as independent variable.

Results: In categorical comparison, we found 619 DMPs/66 DMRs. Among TOP10 DMRs, we identified a region mapping to *DHX30* associated with neurodevelopmental disorders. No enrichment was identified. In continuous comparison, we found 38 DMPs/4 DMRs. We identified a region mapping *CERS3* that was found to be differentially methylated in brains of schizophrenia patients. We found the following GO pathways: glycoprotein metabolic process, glycosylation, presynapse and dendrite. We also identified the neuronal system REACTOME pathway. However, only the first two GO pathways remained significant after BH correction.

Conclusions: Our study suggests an association of DNA methylation and PS using two different comparisons (categorical and continuous). We found an enrichment of several pathways related with brain, that supports previous studies that suggested psychiatric disorders to have an early neurodevelopmental component.

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P09.123C

A compound heterozygous mutations in *ALDH7A1* at pyridoxine-dependent epilepsy (PDE): a case report

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PDE(MIM:266100) is autosomal recessive disease caused by mutations in the gene *ALDH7A1*. The main criteria of PDE is the response to admission of pyridoxine, resistance to treatment by anti-epileptic drugs. Here we report the clinical case. Newborn, on first day of life developed tonic-clonic convulsions. Negative response to initial pyridoxine admission. Later was polymorphism of convulsive seizures: focal clonic, epileptic spasms, myoclonic seizures. Correction of anticonvulsant therapy was no effect. In dynamics, the frequency and severity of seizures increased to appearance of epileptic status. CMA&Karyotyping did not reveal abnormalities. Molecular genetic study for proband and his mother revealed heterozygous variants: (1)NM_199037.4: c.769G>A(*SCN1B*)(p.Gly257Arg); (2)NM_001182.4: c.1279G>C(*ALDH7A1*)(p.Glu399Gln); (3)NM_001182.4: c.328C>T(*ALDH7A1*)(p.Arg82Ter). An unaffected mother has only 1&2 variants. Presently, girl 3y/o delays in

psychomotor development: she fixes the sight for a short time, turns on its side, does not sit, not crawl, not walk, not babbling. Individual attacks persist despite daily intake of B6 and P-5-P. We suppose the increased resistance to primary pyridoxine administration could be partly owing to variants in both, *ALDH7A1* and *SCN1B*. Compound heterozygous in *ALDH7A1* gene causes PDE, while *SCN1B* variant could contribute to severity of the proband condition, complicating the PDE recognition by routine manner after birth (in literature the same compound heterozygous mutations were described for Dutch boy 6y/o only). It should be noted the clinical picture of proband's seizures was uncharacteristic for *SCN1B* mutations. However, the quantitative assessment of individual gene contribution still remains unclear. The authors are grateful to MD Zhylina S. for help.

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P09.124D

Low penetrance in *RANBP2*-related autosomal dominant acute necrotizing encephalopathy (ADANE)

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Autosomal Dominant Acute Necrotizing Encephalopathy (ADANE) is a recently described condition determining frequent severe sequelae after an acute encephalitis most commonly occurring after a symptom-free interval of several years. Described in 2009 by Neilson, only less than sixty patients have been described until now, either sporadic cases or small families with two or three affected patients. Only two large pedigrees are reported in literature, with an intriguing lack of penetrance in most relatives with large sibships of unaffected individuals and occasional index patients. We described another large pedigree with two severly affected children aged 6 and 9 years at the time of presentation and important post-critic encephalopathy with a frontal syndrome in both. Interestingly, some relatives were heterozygous for the c.1754C>T (p.Thr585Met) *RANBP2* familial mutation without manifesting any clinical symptom, except seizures in two, developmental delay or learning disability in one and an acute pseudo-Quincke facial and laryngeal edema in one. In addition 4 histories of meningitis in infancy were noted, without any proof that these episodes occurred in mutated individuals. In summary, this condition thought to be determined by an uncontrolled inflammatory process remains apparently triggered by environmental or epigenetic factors at large making it a singular condition among mendelian disorders. Further studies are required to elucidate the underpinning mechanisms. Interestingly, autosomal dominant transmission mode has been demonstrated in three large pedigrees with a high percentage of non-penetrance.

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P09.125A

Identity-by-descent mapping and whole-exome sequencing implicates neuronal development pathways in schizophrenia and bipolar disorder

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Background: Hundreds of common alleles have been implicated in schizophrenia (SCZ) and bipolar disorder (BPD), but recently a role for rare, high-penetrant variants has been also suggested in both disorders.

Methods: This study investigated a familial cohort of SCZ and BPD patients from a closed population, where the high recurrence of the disorders indicated a possible enrichment in rare risk alleles. A total of 230 subjects were genetically investigated through a strategy that integrated identity-by-descent (IBD) mapping and whole-exome sequencing (WES).

Results: IBD analysis allowed to track high risk haplotypes shared exclusively by patients from different families and possibly carrying the most penetrant alleles. A total of 444 non-synonymous sequence variants, of which 137 disruptive, were identified in these haplotypes by WES. Interestingly, gene sets previously implicated in SCZ (i.e. post-synaptic density (PSD) proteins, voltage-gated calcium channels (VGCCs) and fragile X mental retardation protein (FMRP) targets) were significantly enriched in genes carrying these rare variants. Further, IBD variants were preferentially affecting genes involved in the extracellular matrix (ECM) biology and axon guidance processes.

Conclusions: Results confirmed rare risk variants as key factors in SCZ and BPD pathogenesis and highlighted an involvement of ECM biology and development of neuronal projections in the etiology of both disorders.

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P09.126B

Allele-specific X chromosome inactivation in Rett syndrome patients

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Introduction: Rett syndrome (RTT; MIM#312750) is a severe neurological disorder which mainly affects young females and is the second most common cause of severe intellectual disability in women worldwide. In most cases, it is caused by mutations in *MECP2* (MIM*300005), the gene encoding MeCP2 (methyl CpG binding protein 2), which is located on the X chromosome. Many studies have been carried out to assess whether X chromosome inactivation (XCI) plays a role in the wide range of phenotypic variation of these patients. However, classical methylation-based protocols to evaluate XCI were only able to determine whether the XCI pattern was biased or random, but not if the preferentially inactivated X chromosome was the one carrying the mutant or the wildtype allele.

Materials and Methods: We have developed an allele-specific methylation-based assay to evaluate methylation on the loci of several recurrent *MECP2* mutations in blood samples of RTT patients.

Results: The aim of this study is to provide data to effectively correlate XCI patterns to the phenotypic presentation of RTT.

Conclusions: If this correlation is strong enough, it could be used in the future as a molecular tool to predict the severity of the clinical presentation of genetically diagnosed RTT patients.

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P09.127C

Rett syndrome: Correlation between clinical, molecular and QTc evaluation in 21 patients

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Introduction: Rett syndrome is a neurodevelopmental disorder that affects mostly females and is related, in the majority of cases, to *MECP2* mutations. It is reported that affected patients have a higher probability of sudden death mostly due to a prolonged corrected QT interval (QTc).

Materials and Methods: We included 21 patients diagnosed with Rett syndrome, 19 of those tested for *MECP2* mutations. The patients' medical notes were analysed and used for the classification of their clinical forms and their clinical findings described. The patients were also submitted to electrocardiogram (ECG) evaluation for QTc measurement, which were considered prolonged when >450ms, and their risk for Long QT Syndrome (LQTS) was calculated.

Results: The classic clinical form was more prevalent in our cohort (85%). In the clinical findings, the mean age was 18 years old. Stereotypical hand movements, epilepsy, and bruxism were the most prevalent symptoms (80%, 76%, and 76% respectively). The average QTc was 401ms and prolonged QTc was found in 1 patient. However, none were taking medications known to prolong QTc and none had a higher risk for LQTS.

Conclusions: The classic form of Rett syndrome prevails in our cohort and in most of the cases, the management of their seizures required medication. We found no correlation between prolonged QTc and *MECP2* mutations in our study. None of the patients investigated exhibited a high risk for LQTS. Nevertheless, a follow-up with ECG is highly recommended.

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P09.128D

Characterization of large deletions of the *MECP2*gene in Rett syndrome patients by gene dosage analysis

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Introduction: Rett syndrome (RTT) is a neuromaintenance disease that affects 1:12000 newborn girls becoming the second cause of mental retardation in women after Down syndrome. In more than 96% of the cases of classic RTT a mutation affecting the *Methil-CpG-binding protein 2* (*MECP2*) has been found and in ~15% of the cases, the alteration is a big deletion within it. We carried out the characterization of the break points of the deletions found in 17 classical RTT patients.

Materials and Methods: MLPA was performed in all of them to detect the alteration. Then, the allele containing the deletion was narrowed down via DNA-qPCRs and long-PCRs until Sanger sequencing of it could be done.

Results: Following this methodology we could confirm the presence of the deletion in every case and determine the area, sometimes even the exact nucleotide, where the large rearrangement has occurred. Further analysis of the sequences surrounding the break points showed that most of them happened in regions full of repetitive elements such as *Alus*.

Conclusions: We therefore provide more evidence to support this former theory regarding the possible cause of these rearrangements. Besides, the X chromosome inactivation pattern was determined and along with clinical data a possible correlation between genotype and phenotype has been searched.

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P09.129A

The most recurrent monogenic disorders that overlap with the phenotype of Rett syndrome

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Introduction: Rett syndrome (RTT) is an early-onset neurodevelopmental disorder that is caused by mutations in *MECP2*, but defects in a handful of other genes (*CDKL5* and *FOXG1*) can lead to presentations that resemble classical RTT, but do not completely identical. Here, we attempted to identify other monogenic disorders that share features with RTT.

Material and Methods: It has been studied 396 patients with Rett-like clinical diagnosis. It has been performed: 242 patients by custom panel with 17 gens related to Rett-like clinic through *HaloPlex Target Enrichment System* and 154 patients by commercial panel, *TruSightOne Sequencing Panel*.

Results: 35 patients had Rett-like clinical features and pathogenic variants have been found in six different genes: eleven in *STXBP1* (Epileptic encephalopathy, early infantile,4. OMIM#612164), nine in *TCF4* (Pitt-Hopkins syndrome. OMIM#610954), six in *SCN2A* (Epileptic encephalopathy,early infantile,11. OMIM#613721), four in *MEF2C* (Mental retardation. OMIM#613443), three in *SYNGAP1* (Mental retardation. OMIM#612621) and two in *KCNQ2* (Epileptic encephalopathy,early infantile,7. OMIM#613720).

Conclusions: The genetic study by NGS allows to study a larger number of genes associated with Rett-like clinic simultaneously, providing a genetic study to a wider group of patients. These variants identified by NGS may modify the initial clinical diagnosis to other neurodevelopmental syndromes, or determine new candidate genes related to RTT-like symptoms, providing the clinician with more information and clues that could help in the prevention of future symptoms or in the pharmacologic therapy.

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P09.130B

Mutation screening and global gene expression analyses of

Saudi Rett patients implicate mitochondrial dysfunction in the pathogenesis of Rett syndrome

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Rett syndrome (RS) is a rare neurodevelopmental disorder that is found in a variety of racial and ethnic groups with a notable female predilection. *MECP2* mutations are particularly known to cause RS phenotype, however, involvement of other genes has also been reported. We herein present a detailed molecular characterization of a cohort of 32 patients with RS Mutation screening revealed that 13 patients had *MECP2* mutations, three of which were novel. One patient had a novel *FOXG1* mutation and another one had a novel *CDKL5* mutation. All patients were females except one male who carries *FOXG1* mutation. Patients screened for cytogenetic abnormalities did not carry any gross chromosomal abnormality except for singleton who had a gain on chromosome X harboring two genes including *MECP2*. Whole-transcriptome analysis of RS patients with *MECP2* mutation and sex- and age-matching controls revealed alterations in a number of mitochondria related pathways, including oxidative phosphorylation and mitochondrial dysfunction. We sequenced mtDNA on the patients who did not have any tested genetic defect which revealed two novel mtDNA alterations in two patients. Furthermore, we identified common genes and pathways possibly leading to autistic phenotype by performing network analysis of RS significant genes with the multi-disorder autism geneset that is associated with autism and at least one of 13 other autism sibling disorders. The gene signatures and novel alterations that were found in this study along with the observed disturbance of the expression of mitochondrial pathways may indicate the involvement of mitochondria in the Rett disease pathogenesis.

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P09.131C

Implications of an admixed Brazilian population in Schizophrenia polygenic risk score

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The Polygenic Risk Score (PRS) tool compiles data from hundreds to millions of common variants into a single measure, making it a valuable tool to investigate genetic risk of complex diseases, like Schizophrenia (SCZ). To calculate the PRS-SCZ, a reference sample must be defined, but most subjects from such samples are Caucasian and doubts remain about the reliability of PRS-SCZ in admixed samples. We verified if PRS-SCZ could differentiate patients with schizophrenia and healthy controls in a Brazilian sample and if miscegenation could influence the results. We used the Psychiatric Genomics Consortium-SCZ summary statistics GWAS as reference. As target sample, we genotyped 177 patients with schizophrenia and 242 healthy controls. The Sanger Imputation Service platform was used to impute genomic regions. To perform the quality control and to generate the PRS we used PRSice and PLINK software. We could explain up to 5.2% of the variance between cases and controls when including all individuals. A similar result was found when selecting only individuals with large African component mixed with Caucasian and Native Amerindian components (named as Latin 1). Considering only Caucasians, the variance explained raised to 11%, with the PRS-SCZ significantly higher in patients with SCZ than controls. The same was observed in a sample with large Native Amerindian component mixed with Caucasian component (named as Latin 2). We found more robust results after restraining the sample to Caucasian subjects, but PRS-SCZ was still capable to differentiate cases from controls even in a highly mixed population.

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P09.132D

Genome wide DNA methylation analysis in a longitudinal cohort of antipsychotic naive first episode of psychosis patients

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Identifying the genetic and molecular changes of drug response is the first step through personalized medicine. In this study we aimed to identify DNA methylation markers in blood of an antipsychotic-naive First Episode of Psychosis (anFEP) cohort before and after two months of risperidone treatment (FEP-2M), furthermore, we investigated overlaps between these markers and *post-mortem* schizophrenia brain datasets. Sixty anFEP were recruited for this study. We used the Human_Illumina-450K microarray. We covariated the data for sex, age, smoking and cell proportions, considering as statistically significant differentially methylated positions (DMPs) with a p-value < 0.05 after a FDR and as differentially methylated regions (DMRs) those with at least two DMPs. E-GEOD-61107, E-GEOD-61380 and E-GEOD-61431 brain datasets were used to investigate for DMP overlaps between brain and blood. We searched for enriched pathways using the WebGestalt software. We found 14 DMRs between anFEP and FEP-2M, most related to treatment response (*ANKRD33*) or side effects such as male fertility (*BRDT, TEX14, ASZ1, STARD6*) and metabolism (*CPN1, ERLIN1, ASB3*). Comparing results with brain datasets, we found 27 DMPs overlap in RNF39 gene, which lies within the MHC region. No biological pathway were enriched for blood or brain studies. To our knowledge, this is the first study to find DMPs and DMRs in a longitudinal cohort of anFEP. Collectively, we identified DMRs that seem to be related to the adverse effects of risperidone and a large overlap between blood and brain studies close to the most associated genomic region in schizophrenia, the MHC region.

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Polygenic risk score analyses of symptoms and treatment response in an antipsychotic-naïve first episode of psychosis cohort

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In this study, we aimed to test if the schizophrenia (SCZ) polygenic risk score (PRS) was associated with clinical symptoms at: a) the first episode of psychosis pre-treatment (FEP), b) nine weeks after initiation of risperidone treatment (FEP-9W) and c) with the response to risperidone. We performed a detailed clinical assessment of 60 antipsychotic naïve patients in their FEP and, again, after nine weeks of standardized treatment with Risperidone. Blood derived DNA was genotyped using the Illumina PsychArrayChip, along with 59 controls, and then imputed. We used the latest available GWAS summary statistics from the Psychiatric Genomics Consortium wave-2 SCZ group as a training set to calculate their PRS for schizophrenia. We used Poisson Regression to test association between the PRS and clinical measures adjusting for four ancestry principal components. We considered as significant a p-value < 0.001 (Bonferroni correction). First, we verified that the schizophrenia PRS was also able to distinguish cases from control in this south-eastern Brazilian sample, with a similar variance explained (~0.19, observed scale) to that seen in Northern European populations. In addition, within-cases, we found that PRS is significantly positively correlated with baseline (pre-treatment) symptoms as measured by the PANSS-excitement factor. After standardized treatment for 9 weeks, this correlation disappeared and the depressive symptoms (CDSS) became negatively associated with PRS. These results highlight the importance of studying schizophrenia, and other disorders, pre-treatment to understand the relationship between polygenic risk and phenotypic features.

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P09.134B

A molecular analysis of SDCCAG8, a schizophrenia risk gene that functions in the centrosome

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Schizophrenia affects 1% of adults and is a major global health problem. The focus of my project is the potential role of the centrosome in schizophrenia. The centrosome, an organelle within cells, plays a crucial role in brain development where it directs cell shape, polarity and motility. The centrosome also seeds the growth of antenna-like signalling structures called primary cilia. Rare mutations in centrosome genes cause disorders that present with severe cognitive deficits and variable neuropsychiatric phenotypes.

GWAS data has implicated many genes in schizophrenia. We have shown that seven schizophrenia risk genes encode proteins with centrosomal functions. Of these, SDCCAG8 is also associated with educational attainment in GWAS and the genome-wide significant SNPs for the two phenotypes are in high linkage disequilibrium indicating a pleiotropic effect. We have found that a schizophrenia risk SNP in SDCCAG8 is significantly associated with poorer performance in a social cognition task, in a large Irish dataset of schizophrenia patients and controls ($p = 0.001$).

To analyse the molecular function of SDCCAG8 we have used genome editing to knock it out in neuronal and retinal cells. Loss of SDCCAG8 impairs cells' ability to make primary cilia and their capacity to repair genome damage. Nuclear lobulation has also been observed. Current work is addressing whether SDCCAG8 affects cell signalling using RNA-Seq analysis. This could identify molecular mechanisms by which SDCCAG8 mutations contribute to schizophrenia risk and cognition and help uncover the processes that implicate centrosome genes in neurodevelopmental phenotypes.

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Targeted WES study, an effective tool for the genetic diagnosis of epilepsy

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Background: Epilepsy is a common clinical and genetic heterogeneous neurological disorder, with a large number of cases caused by genetic factors. To understand the molecular basis of epilepsy, 234 epileptic patients were studied using a targeted sequencing of 223 epilepsy-associated genes.

Material and Methods: We performed exome sequencing using the Ion AmpliSeqTM Exome RDY, combined with an AmpliSeq panel design and SureSelectXT technology. Sequencing reads were analyzed using Torrent Suite software and an in-house pipeline, respectively. Annotated variants using ION Reporter were prioritized with an in-house analytical pipeline.

Results: Among the 234 cases, 152 patients were referred as Early Infantile Epileptic Encephalopathy (EEIE). On this group a diagnostic yield of 35% was obtained. *SCN1A*, *CDKL5 KCNT1, KCNQ2*, and *SPTAN1* were the most frequently mutated genes. On the 82 remaining patients, 33% of them with an associated neurodevelopmental disorder, potential diagnostic variants were detected in 25% of the cases, being *IQSEC2*, identified in 3 cases, the only recurrent mutated gene on this group. Out of the 281 identified variants, 171 (61%) were associated to autosomal-dominant inheritance pattern diseases. Variants of uncertain significant category were identified in *RYR3, ARHGEF15, FASN* and *RELN* genes. Recurrent updating of targeted genes and the familial segregation studies has shown to be essential to identify causal variants.

Conclusions: Targeted sequencing based on whole exome sequencing in epilepsy patients provide a cost effective and comprehensive strategy that accelerates the identification of a definitive clinical diagnosis on EEIE and on patients with seizures associated to other neurological disorders.

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High incidence of *SHANK3* loss of function mutations in individuals with intellectual disability and autistic traits

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SHANK3 deletions or loss of function mutations cause Phelan-McDermid syndrome and have been found in 2% of individuals with intellectual disability (ID) and 0.5% of individuals with autism spectrum disorders (ASD). We analyzed *SHANK3* coding sequence (NM_033517; hg19) with amplicon-based next-generation sequencing in 163 individuals, negative to aCGH and Fragile X test, with non-specific ID with or without autistic traits. Due to high GC content, 7% of the gene was not covered by the analysis. We identified six novel *SHANK3* variants, of which four de novo frameshift or nonsense mutations. Individuals carrying truncating mutations had global developmental delay with moderate to severe ID, severely delayed or absent speech, and abnormal behavior. Although some features were present, ASD was specifically referred only for one of them. The other two variants were missense with discordant prediction of pathogenicity; one inherited from an unaffected parent was found in a girl with Rett-like phenotype. The other missense, apparently homozygous, was found in a boy with ID, ASD, epilepsy, speech delay and macrocephaly, who carried other four apparently homozygous *SHANK3* variants. We are currently investigating the possible presence of a previously missed intragenic deletion. The higher incidence of *SHANK3* mutations (2,5%) we report in individuals with ID and autistic traits indicates *SHANK3* haploinsufficiency as one of the most prevalent monogenic causes of ID and ASD. We suggest routine screening of *SHANK3* for the diagnosis of non-specific ID in individuals with or without reported autistic traits. Funding: Italian Ministry of health Young Investigator Grant GR-2011-02347754 to E.L.

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P09.138B

Serotonin transporter polymorphism, cortisol and hippocampal volume in post-stroke patients

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Background: The s allele variant of the serotonin transporter gene (5-HTT) has been related to hypothalamic-pituitary-adrenal (HPA)-axis reactivity to stress, depressionand negatively impact on memory. Acute strokeis associated with elevated cortisol levels as part of the body's reaction to a stress provoking event. We investigated whether 5-HTT genotype interacts with physiological stress to impact on cognitive function and hippocampal structural measures in stroke patients.

Methods Data from 182 cognitively intact stroke patients from the TABASCO study were available. Patients underwent 3T MRI scans, saliva cortisol measure and comprehensive cognitive and depression assessments at admission, 6, 12 and 24 months thereafter.

Results Carriers of the 5-HTT s allele had significantly higher admission bedtime salivary cortisol and reduced hippocampal volume than non-carriers. Patients with smaller hippocampi had lower cognitive scores at all timepoints post-stroke, and higher depression scores. Carriers of the 5-HTT s allele displayed strong negative association of admission cortisol with hippocampal volume and cognitive scores at all timepoints, while non-carriers displayed no such association.

Conclusions Carriers of the 5-HTT s allele had lower hippocampal volume and higher admission salivary cortisol . Their cortisol levels negatively correlated with post-stroke cognitive function. . The interactive effects of the s allele and cortisol levels on reduced hippocampal volume,lower cognitive scores and higher depression imply that the negative effect of 5-HTT-s on cognition involves the HPA axis. Since genetic factors may influence vulnerability to the adverse effects of stress, serotonin receptors may provide a novel target for therapeutics to prevent dementia in stroke patients.

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SINEUP, a synthetic antisense non-coding RNA-based technology, as possible new therapeutic tool for haploinsufficiency: Autism Spectrum Disorders (ASD) and Epilepsy as Proof-of-Principle

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Autism spectrum disorders (ASD) and epilepsies are heterogeneous conditions that frequently coexist with other developmental disabilities. Genetic bases are prominent risk factors for both disorders. Among others, loss of function mutations in *CHD8* gene represents a recurrent risk factor for ASD, while *CHD2* is more frequently mutated in epilepsy. Thus, the sole reduction in *CHD8* or *CHD2* expression is able to cause cellular and molecular phenotypes that are key hallmarks to follow and rescue in assessing new therapeutic approaches.

Particularly, we aim to test SINEUPs, a novel class of synthetic antisense long non-coding RNAs - able to increase the translation of target proteins to physiological level without affecting transcription - to rescue the phenotypes caused by *CHD8* or *CHD2* haploinsufficiency.

Since the activity of SINEUP depends on two domains, an effector domain required for translation enhancement and a binding domain conferring target specificity, we designed SINEUP molecules able to recognize the initial and internal methionines of CHD8 and CHD2 proteins. We then proceeded to test the efficacy of different SINEUPs on neural progenitors. From our preliminary observations, SINEUPs targeting internal methionines are more efficient in stimulating CHD8 and CHD2 protein production, thus representing a valid target to be further tested in patients' derived cell lines and in zebrafish, an *in vivo* model of the disorders.

In conclusion, our studies represents the first step towards the development of new types of RNA-based therapy, with implications for a large repertory of presently incurable genetic diseases.

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P09.141A

Delineation of *SPATA5* related epilepsy, hearing loss, and mental retardation syndrome (EHLMRS)

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Bi-allelic variants in *SPATA5* (spermatogenesis-associated protein 5, MIM: 613940) are associated with severe global developmental delay, congenital sensorineural hearing loss, seizures, cortical visual impairment and microcephaly. SPATA5 is vital for mitochondrial function and morphology in the cortical neurons. The absence of functional protein prevents the normal neuronal development and interferes with axonal growth. Congenital sensorineural hearing impairment is often the first presenting symptom, followed by seizures and motor delay on the back ground of abnormal neurological phenotype including core hypotonia, increased peripheral tone. A slowly progressive hyperkinetic movement disorder evolves from early childhood. Most patients have microcephaly, although brain imaging is non-specific, demonstrating brain atrophy and/or delayed myelination. In partnership with the patient support group, we have had access to an international cohort of patients with confirmed *SPATA5* mutations. We provide a detail clinical description of the breadth and variability of the clinical phenotype, alongside the already reported cases in the literature. *SPATA5* should be considered in cases suggestive of mitochondrial disorders especially in young infants whose clinical picture is often less recognisable.

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Biallelic *SQSTM1* mutations in early-onset, variably progressive neurodegeneration

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A. Jeffries[12], J. Zeighami[7], A. Sherafat[13], D. Di Giuda[14], G. Shariati[7], R. Carrozzo[1], N. Katsanis[3], R. Maroofian[9], S. Servidei[15], M. Tartaglia[1]

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Intracellular clearance of damaged cellular constituents, including protein aggregates and dysfunctional organelles, is necessary for proper neuronal function and long-term survival of neuronal cells. Autophagy contributes significantly to this process, and its defective function has been implicated in a number of neurodegenerative disorders. Here, we describe clinically and molecularly a recently recognized early-onset, variably progressive, neurodegenerative disorder caused by loss of function of SQSTM1, a multidomain protein serving as a selective autophagy receptor. Eleven affected individuals from three consanguineous families shared a homogeneous phenotype characterized by ataxia, hypotonia, dysmetria, dysarthria, ophthalmoplegia, dyskenesia, and cognitive decline as major features. Whole exome sequencing (WES) in two families, and a combined approach based on homozygosity mapping analysis in six affected individuals of the third family coupled to WES performed in a single affected member allowed to identify three homozygous inactivating variants, including a splice site substitution (c.301+2T>A) causing aberrant transcript processing and accelerated degradation of a resulting protein lacking exon 2, and two truncating changes (c.875_876insT and c.934_936delinsTGA). *In vitro* studies directed to characterize the consequences of loss of SQSTM1 function on autophagy provided evidence of a decelerated autophagic flux and impaired production of ubiquitin-positive protein aggregates in response to misfolded protein stress. The impact of sqstm1 down-modulation on the structural integrity of the cerebellum was analyzed *in vivo*, using zebrafish as model, documenting a variable but reproducible phenotype characterized by cerebellum anomalies ranging from depletion of axonal connections to complete atrophy. Italian Ministry of Health (R. C. 2017)

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P09.143C

Compound heterozygosity for a frameshift and a missense mutation in the *SURF1* gene in a patient without Leigh syndrome

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The *SURF1* gene encodes an assembly factor of the mitochondrial respiratory complex IV. Recessive mutations in the *SURF1* gene are associated with Leigh syndrome (MIM 256000) and a few patients with Charcot-Marie-Tooth disease type 4K (MIM 616684). The majority of the reported mutations lead to premature termination codons. Missense mutations are reported to cause a milder phenotype and longer survival. Here we report an adult patient showing episodes of balance and coordination problems, tremor, short stature and stuttering who was referred to our clinic for whole exome sequencing. We identified compound heterozygosity for a missense and a frameshift mutation in the *SURF1* gene. Both mutations have previously been reported in patients with Leigh syndrome, however, the clinical phenotype of the patient did not fit Leigh syndrome. The clinical phenotype of our patient and those reported with the same mutations is compared and will be presented. Overall, mutations in the *SURF1* gene comprise a broad clinical phenotype and should also be considered in adult patients with movement disorders.

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P09.144D

Natural history of a 34 year old male with an apparently balanced translocation t(1,17)(q24,q24.2) disrupting the BPTF gene

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Introduction: We present a 24-year follow-up of a 34-year-old male with a *de novo* apparently balanced chromosomal translocation t(1;17)(q31;q25) reported in 1993 with the features of Russell-Silver syndrome (RSS, PMID: 8403458). The chromosomal region 17q25 was also involved in another apparently balanced translocation t (17;20)(q25;q13) associated with RSS (PMID: 1633648). Molecular studies indicated a disruption of *KPNA2*. However, subsequent screenings for mutations in *KPNA2* in 31 unrelated individuals with RSS revealed no disease-related variants (PMID: 11735022).

Material and Methods: Phenotypic analyses were performed according to the Munich Dysmorphology Database (MDDB) methodology. Chromosomal microarray analysis and FISH with BAC and fosmid clones were used to narrow the 17q breakpoint.

Results: We found that the translocation breakpoint maps to 17q24.2 and disrupts *BPTF* encoding the largest subunit of a nucleosome remodeling factor (NURF), a member of ISWI chromatin remodeling complex. No non-polymorphic CNVs were identified. Phenotypic analyses showed short stature, hemihypotrophy, microcephaly, triangular shape of face, prominent forehead, hypertelorism, protruding eyeballs, broad palpebral fissures, long eye lashes, long nasal bridge, short philtrum, thin lips microretrogenia, and bilateral 5th finger clinodactyly. Muscle hypotonia, intellectual disability, vision problems, speech delay, and the defect of phonemic audition have improved during 24 years of treatment.

Conclusion: Observed phenotypic changes are similar to those seen in the recently described patients with Neurodevelopmental disorder with dysmorphic facies and distal limb anomalies (NEDDFL, OMIM 617755) due to haploinsufficiency of *BPTF*, further demonstrating its pathogenicity. Our data expand the clinical spectrum of human disorders caused by ablation of chromatin remodeling complexes.

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P09.145A

Systematic analysis of the involvement of DNA tandem repeats in Amyotrophic lateral sclerosisfrom Whole Genome Sequencing data

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The C9ORF72 gene repeat expansion is the most frequent cause of amyotrophic lateral sclerosis (ALS). Long repeats alleles in ATXN-1, ATXN-2, and NIPA1 genes are associated to ALS susceptibility. Thus, Tandem Repeat Polymorphisms (TRPs) are good candidates for missing hereditability in ALS, although they were never systematically analyzed because challenging to NGS. The general aim of this study is to perform a systematic analysis of TRPs in ALS by combining NGS and novel bioinformatics tools. TRPs from whole genome sequencing data (WGS, Illumina HiSeq X Ten, avg. coverage 30X, 2x 150 bp length) of a cohort of 70 ALS cases enriched in FALS cases were evaluated by means of two software developed within our consortium and by a literature software (lobSTR) to detect either expansions with a repeat size within the NGS reads sizes (short TRPs), and repeat expansions larger than the NGS reads sizes. The analysis of short tandem repeat expansion for about 600K loci in 70 ALS cases and 300 controls led to the selection of 20 TRPs showing a significant distribution among patients and controls. The validation of these loci by traditional methods revealed a high technical consistency (70%). However, we failed to replicate this data in an independent sample (208 Italian ALS patients and 229 matched controls). For large repeat expansions detection, the analysis of 700K TRPs identified 16 loci with potential very large repeat expansion observed in 1 or 2 of the 70 patients, and whose validation and replication is ongoing.

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P09.146B

Generation and in-depth characterization of 20 induced pluripotent stem cell (iPSC) lines from 10 dystonia patients and healthy carriers of *THAP1* mutations

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Introduction: Mutations in *THAP1* have been linked to dystonia (DYT-THAP1, DYT6) with reduced penetrance. *THAP1* encodes a transcription factor that regulates its own expression and the expression of *TOR1A*, another dystonia gene. To date, little data is available on the expression of *THAP1* and *TOR1A* in mutant THAP1 induced pluripotent stem cells (iPSCs).

Material and Methods: Cultured skin fibroblasts were reprogrammed into iPSCs using Sendai virus. Two clones per patient were comprehensively characterized by testing for the mutation using Sanger sequencing, by expression analysis of four pluripotency markers using quantitative PCR and immunocytochemistry, and by their ability to differentiate into all three germ layers. Genomic rearrangements were excluded by single nucleotide polymorphism (SNP) array analysis. Expression of *THAP1* and *TOR1A* was tested by quantitative PCR compared to 10 iPSC controls while *ß-Actin* served as a reference gene.

Results: We generated 20 iPSC lines of 10 affected and unaffected members of three families carrying pathogenic *THAP1* variants (p.Arg13His [4 lines], p.Ser21Cys [10 lines], p.Leu159fs180X [6 lines]). *THAP1* expression was reduced for Ser21Cys and *TOR1A* expression in Arg13His and Ser21Cys mutant cell lines compared to controls.

Conclusion: We report the generation and characterization of 20 lines from 10 human THAP1 iPSC lines as well as alterations in *THAP1* and *TOR1A* expression in these cells. These stem cells can further serve as an ideal model to investigate the mechanism of reduced penetrance by transcriptomic analysis in affected and unaffected THAP1 mutation carriers on the stem cell and differentiated neuron level.

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P09.147C

***TOR1A* variants cause a severe arthrogryposis with developmental delay, strabismus and tremor**

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Abstract

Background: Autosomal dominant torsion dystonia-1 is a disease with incomplete penetrance most often caused by an in-frame GAG deletion (p.Glu303del) in the endoplasmic reticulum luminal protein torsinA encoded by *TOR1A*.

Methods: We report an association of the homozygous dominant disease-causing *TOR1A* p.Glu303del mutation, and a novel homozygous missense variant (p.Gly318Ser) with a severe arthrogryposis phenotype with developmental delay, strabismus and tremor in three unrelated families.

Results: All parents who were carriers of the *TOR1A* variant showed no evidence of neurological symptoms or signs, indicating decreased penetrance similar to families with autosomal dominant torsion dystonia-1. The results from cell assays demonstrate that the p.Gly318Ser substitution causes a redistribution of torsinA from the endoplasmic reticulum to the nuclear envelope, similar to the hallmark of the p.Glu303del mutation.

Conclusion: Our study highlights that *TOR1A* mutations should be considered in patients with severe arthrogryposis and further expands the phenotypic spectrum associated with *TOR1A*.

Keywords: *TOR1A*; Endoplasmic reticulum luminal protein torsinA; DYT1 dystonia; *TOR1A* p.Glu303del; Severe arthrogryposis

A. Kariminejad: None. **M. Dahl-Halvarsson:** None. **G. Ravenscroft:** None. **F. Afroozan:** None. **E. Keshavarz:** None. **M. Faraji Zonooz:** None. **H. Najmabadi:** None. **H. Goullée:** None. **M. Davis:** None. **N. Laing:** None. **H. Tajsharghi:** None.

P09.148D

Identification of a third family further confirms TRAPPC6B as a disease gene associated with significant neurological dysfunction

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Increasingly whole exome sequencing (WES) is used as an early diagnostic tool in patients with undiagnosed rare diseases. Particularly in the setting of consanguinity, hundreds of emerging disease genes, usually with private mutations, have been identified. Typically, these require replication to validate as bonafide disease genes to facilitate management of such families. The transport protein particle (TRAPP) family of protein complexes regulates intracellular trafficking between the endoplasmic reticulum and Golgi apparatus. Variants in several TRAPP subunits have been implicated in diverse human diseases. Harripaul (2017) reported a homozygous nonsense variant in one such subunit, *TRAPPC6B*, in two consanguineous individuals with non-syndromic intellectual disability. A homozygous founder splice variant in *TRAPPC6B* was recently described by Marin-Valencia (2018) in 3 Egyptian sibships with microcephaly, global developmental delay, autism, and epilepsy (OMIM 617862). We report two consanguineous Pakistani sisters with microcephaly (-8 SDs) and severe global developmental delay. Additionally, the younger sister had a movement disorder while the older sister had epilepsy. Trio WES of the parents and younger sister revealed a homozygous novel variant in *TRAPPC6B* at a splice donor site (c.149+2T>A), carried by both parents. This variant is predicted to cause skipping of exon 2, and has been reported once, in heterozygous form, in gnomAD. Therefore, we report the third "family" with homozygous *TRAPPC6B* variants. These findings support the role of recessive mutations in *TRAPPC6B* in severe disease. We will review the phenotype associated with recessive mutations in *TRAPPC6B* as well as those associated with other members of the TRAPP family.

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P09.149A

Functional characterization of a new *TTBK2* missense variant: uncovering the molecular basis of SCA11

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SCA11 is a rare autosomal dominant form of cerebellar ataxia, characterized by early-onset cerebellar ataxia and nystagmus. SCA11 is caused by variants in *TTBK2;* the ones reported are heterozygous truncating variants. Nevertheless, the disease mechanism linking *TTBK2* and SCA11 remains unclear. *TTBK2* encodes tau tubulin kinase 2 protein, a protein kinase involved in different cellular processes, namely, ciliogenesis, microtubule dynamics, and tau and TDP-43 phosphorylation.

Our group has previously identified a novel heterozygous missense variant in *TTBK2* in two Portuguese siblings with a diagnosis of ataxia. Therefore, we aim to characterize the potential pathogenic effect of this variant in SCA11. For that, we generated *TTBK2* clones (wild-type and different mutants) in fusion with EGFP-tag that were transfected in cultured cells. The subcellular localization was accessed by immunofluorescence and subcellular fractioning; however, *TTBK2* clones did not display significant changes. TTBK2 kinase activity was evaluated by measuring the phosphorylation state of TTBK2 substrates and potential new interactors of TTBK2 were analyzed by co-immunoprecipitation. Our results showed that the *TTBK2* missense variant impairs phosphorylation activity against TDP-43 and may lead to altered protein-protein interactions, namely with ataxin-2. In addition, we created a cellular model expressing the endogenous *TTBK2* missense variant, by CRISPR/Cas9 technology.

In conclusion, we showed that the newly identified *TTBK2* missense variant confers different biochemical properties, which may result in abnormal protein phosphorylation in SCA11. The study of the novel CRISPR/Cas9 cellular model should contribute to a better understanding of the molecular and cellular mechanisms underlying SCA11.

M. Santos: None. **J. Sequeiros:** None. **I. Alonso:** None.

P09.150B

Severe speech delay in Cohen Syndrome: three novel mutations and the long-term follow-up of nine patients

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Introduction: Cohen Syndrome (CS) is a rare autosomal recessive disorder caused by *VSP13B* mutations. Characteristic features are hypotonia, mild dysmorphic facies at infantile period, microcephaly, retinitis pigmentosa, developmental delay with positive social behavior and intermittent neutropenia in childhood. Here, we investigate the genetic defects and follow-up findings of CS patients.

Materials and methods: Clinical findings of nine patients from five families were evaluated during the follow-up period of 1-14 years. Mutations were identified by using Sanger sequencing of *VSP13B* gene.

Results: While 4 patients were diagnosed at age 3-38 months, 5 patients at 4.5-9.5 years of age. Hypotonia, microcephaly, joint laxity, almond shaped eyes, and micrognathia were present in 4 patients admitted during the infantile period. Patients admitted in childhood had typical facial appearance and microcephaly (5/5), hypotonia (5/5) and also pigmentary retinopathy (2/5), neutropenia (2/5), truncal obesity (2/5) at the first examination. Three novel mutations (1 splice site, 1 nonsense and 1 frameshift) were found. During the follow-up, retinopathy and neutropenia in 2, growth hormone deficiency in 1, hypothyroidism in 2, hyperinsulinemia in 1 patient were detected. Interestingly, severe speech delay has developed in eight patients over 5 years of age.

Conclusions: We aim to contribute to the literature three novel *VSP13B* mutations without any distinct genotype-phenotype relationship. In infantile patients with hypotonia, joint laxity, and typical facial features, CS should be kept in mind. The observation of severe speech delay in our patients over the age of 5 suggests that this finding should be added to diagnostic criteria.

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P09.151C
Analysis of WES data in 15 minutes - new methods override manual filtering

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Introduction: Epileptic encephalopathies are severe, early onset disorders associated with global developmental delay, cognitive dysfunction and ongoing epileptiform activity. A genetic aetiology can be identified in a significant proportion of patients. Whole exome sequencing (WES) analysis by tools like Denovogear and Exomiser has proven to be very effective.

Materials and methods: We performed WES in a cohort of 18 unrelated individuals (9 males and 9 females) with epileptic encephalopathies. All the patients were thoroughly assessed by medical specialists and counselled by a clinical geneticist, in order to provide detailed and complete clinical information. These patients were previously tested by gene panel and the cause of epilepsy was not identified. Ten trios were analysed by Denovogear and eight single samples were analysed with Exomiser also with help of proper HPO terms, OMIM database and HGMD professional database) Manual filtering for all samples was also performed.

Results: After running analyses, we were able to identify pathogenic variants in 28% of patients. Pathogenic variants were found in: *HUWE1*, *UBTF*, *NARS2, PPP2R5D*, *SETBP1*. All these variants were highly prioritised by algorithms and confirmed by segregation analysis in the family. The analysis time per sample was approx. 15 min - instead of hours/days by previous algorithms based on manual filtering. With manual filtering, no other variants of interest were found.

Conclusions: New approaches in WES analysis helps to reduce amount of time spent per sample. With this approach we were able to identify a causal variant in 28% samples.

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P09.152D
NGS gene panels for the challenging diagnosis of neurodegenerative disorders with high genetic heterogeneity

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Introduction: Hereditary ataxias, spastic paraplegias (HSP), white matter disorders (WMD) and peripheral neuropathies (Charcot-Marie-Tooth (CMT) disease) are genetically highly heterogeneous and exhibit phenotypic

overlap. Since >100 genes are known for each group, molecular definition represents a challenging task, with >50% of patients undiagnosed. NGS technology allows a comprehensive and systematic approach for genetic testing.

Methods: We developed and validated different disease-specific gene panels covering >98% of target region at >20X: 1) 205 HSP and ataxia genes; 2) 177 CMT and related neuropathies genes; 3) 143 WMD genes. We analyzed 710 probands (142 HSP, 155 ATAXIA, 332 CMT and 81 WMD) negative for the most frequent forms.

Results: Pathogenic mutations were identified in 25% (178/710) of patients (22% HSP, 21% ATAXIA, 28% CMT and 25% WMD). In particular, we identified mutations in challenging genes difficult to be studied by conventional sequencing because of their length (eg, *SYNE1*, *SACS*, *SETX*, *CACNA1A*). Mutations in extremely rare genes were identified in 10% of cases. Panel design allowed CNV analysis that detected pathogenic CNVs in 13/283 patients and CNV of unknown significance in 9/283 patients. In 10 patients, we identified mutations in genes unexpected based on the clinical diagnosis, thus expanding the phenotypic spectrum. Moreover, pathogenic mutations in more than one gene were identified in 3 patients, thus challenging diagnosis and genetic counseling.

Conclusions: The use of high-coverage panels for the genetic definition of highly heterogeneous neurodegenerative diseases is a reliable (high detection rate, no incidental findings) and cost-efficient approach (Italian MoH-RF-2011-02351165 grant to FT).

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P09.153A

Whole exome sequencing identifies a novel mutation in ADCY5 in a patient with developmental delay and dystonia

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Introduction: Familial dyskinesia with facial myokymia (FDFM) or *ADCY5*-related dyskinesia is an autosomal dominant movement disorder characterized by early-onset of involuntary choreiform or dystonic movements. *ADCY5* belongs to the adenylate cyclase family of enzymes responsible for the synthesis of cAMP. Heterozygous missense mutations in this gene are primarily known to cause the disease. Around 400 cases have been reported in the literature, but the disorder is thought to be underdiagnosed because its features can resemble those of other conditions such as cerebral palsy or epilepsy. In this study, we investigated the cause of neuromotor development delay and dystonia in a 3,5 years old female patient.

Materials and methods: We performed whole exome sequencing in the patient with central hypotonia and facial myokymia. All biochemical and metabolic screening results were normal. Chromosome analysis did not show any abnormalities.

Results: Whole exome sequencing analysis identified a novel heterozygous missense mutation (c.2090G>T; p. Gly697Val) in *ADCY5*. p.Gly697Val occurs in a conserved domain whose function is unknown. *In silico* tools a deleterious effect on the protein. The family study confirmed that it is a *de novo* mutation.

Conclusion: Given its genetic heterogeneity and variable phenotype, molecular diagnosis of dystonia and dyskinesia is difficult. Whole exome sequencing is a powerful diagnostic tool for patients with these phenotypes. Mutations in *ADCY5* should be considered in cases with complex movement disorders and with or without a family history.

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P09.154B

Whole exome sequencing in a cohort of 48 trios with neurodevelopmental disorders

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Introduction: Neurodevelopmental disorders (NDDs) are a heterogeneous group of neurological phenotypes diagnosed during early in life and including epilepsy, brain malformations, autism spectrum disorder, and intellectual

disability. Next generation sequencing increased to over 450 the number of genes associated with NDDs, underling their high genetic and phenotypic heterogeneity. Such a high number of genes as well as genetic and phenotypic heterogeneity make panel based diagnostics unsuitable. In patients with NDDs, analyzed with whole exome sequencing (WES), the molecular yield ranges from 25 to 57%.

Materials and methods: We performed WES in 48 trios with NDDs who were mutation-negative to previous genetic investigations.

Results: We identified pathogenic variants in known disease-genes in 22 patients (22 out of 48: 46%). In three of these patients, we expanded the phenotypic spectrum previously associated with the causative gene. In 9 patients (9 out of 48: 19%), we identified variants in candidate genes (not yet reported as disease-causing genes), possibly explaining clinical symptoms. Finally, in 17 patients (17 out of 48: 35%), we detected variants of unknown significance that were could not be correlated to the clinical condition. In about one-third of all patients the analysis revealed a recessive condition. This was an unexpected result considering the reported excess of *de novo* variants in patients with NDDs.

Conclusions: Our results, although obtained in a small series, indicate WES as a first-line diagnostic option to provide genetic counseling and facilitate personal medical care in NDDs without a clear syndromic picture and characterized by high genetic heterogeneity.

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P09.155C
Fenotype genotype correlations in Italian patients with Wolfram Syndrome 1

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Introduction: Wolfram syndrome 1 (WS1) is a rare, autosomal recessive, neurodegenerative, and progressive disease characterized by diabetes mellitus (DM), bilateral optic atrophy (OA), diabetes insipidus (DI), deafness (D), renal tract or neuropsychiatric abnormalities.

Materials and methods: Genomic DNA has been extracted from 45 Italian WS1 patients (20 males and 25 females) aged 25-45 years. *WFS1* exons have been amplified by PCR and, then, subjected to automatic sequencing. The mutations have been subdivided in 3 groups according to their predicted functional consequences: Group 1 (complete depletion of wolframin); Group 2 (milder degradation of *WFS1* protein than group 1); Group 3 (compound heterozygous mutations not found in 1 and 2 groups).

Results: DM and OA have been found in all patients (100%), DI in 26 (57.7%), D in 27 (60%), renal tract abnormalities in 10 (22.2%), neuro-psychiatric symptoms in 20 (44.4%), and endocrinopathies in 3 (6.6%). Six patients (13.3%) have died of whom 5 for respiratory failure and one for chronic renal failure. We have found 34 different mutations in *WFS1* which were all known except for 2 missense substitutions, c.1523 A>G and c.1514 G>A, both located in exon 8. In 2/45 patients (4.5%), we have detected a mutation of *WFS1* in only one chromosome. We have found 23 patients (54%) in group 1 mutations, 10 (23%) in group 2 and 10 (23%) in group 3.

Conclusions: Although a clear genotype-phenotype correlation is difficult to establish in WS1, we have found several correlations between severe phenotypes and the type of *WFS1* mutations.

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P09.156D
Is microduplication Xp22.31 a possible synergic factor in MECP2 defect for Rett Syndrome?

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Introduction: Rett syndrome (RS) is a neurodevelopmental infant disease characterized by early normal psychomotor development followed by a regression in the acquisition of developmental stages. In the majority of cases, it leads to a sporadic mutation in the MECP2 gene, which is located on the X chromosome. However, this syndrome has also been associated with microdeletion, gene translocations, and other gene mutations. Here we described a classic Rett syndrome associated with Xp22.31 microduplication.

Materials and Method: A Colombian patient who is 12 years old and is a product of quarter gestation, non-consanguinity parents. Initially, her neurological development was normal until 11 months, when she started a seizure syndrome with difficult treatment and regression in the acquisition of developmental stages (especially

language with motor and verbal stereotypes, hyperactivity, and autistic spectrum disorder). Comparative genomic hybridization array-CGH (750K) and MECP2 genes sequence were performed.

Results: Array-CGH detected Xp22.31 duplication (6866889-8115153) with a size of 1.248Mb. Due to clinical criteria of RS a MECP2 gene sequence was performed, which showed de novo pathogenic variant C.338C>G (p. Pro113Arg) associated with this disease.

Conclusion: RS makes a part of the intellectual disability, developmental delay, and autism. These characteristics are associated with copy number variations (CNVs) in the X chromosome such as Xp22. 31 microduplication. This is the first case report in the literature that shows a CNV and MECP2 pathogenic mutation simultaneously in the context of RS. We propose that both DNA alteration might have a synergy effect and could lead to variable expressivity of phenotype.

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P10 Neuromuscular disorders

P10.01A
Whole exome sequencing of patients with amyotrophic lateral sclerosis

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Genetic factors play a key role in ALS and uncovering its genetic background may bring us closer to fully understand its pathomechanism, therefore the aim was to identify rare damaging variants in major and minor genes involved in pathways annotated to ALS.

Patients and methods: The investigated sporadic patients fulfilled the revised El Escorial criteria for ALS. Whole exome sequencing of 21 Hungarian patients affected by ALS was carried out. The patients were prescreened for *C9ORF72* repeat expansion, *SOD1*, *ANG*, *FUS*, *SETX*, *TARDBP* and *UBQLN2* genes. Exome sequencing was performed using Illumina NextSeq sequencer and data analysis was performed according to the best practices to identify single nucleotide variants and small insertions/deletions. The detected variants were confirmed by Sanger sequencing.

Results: Exome sequencing revealed a novel non-synonymous variant (T338I) in *NEFH* gene that encodes neurofilament heavy polypeptide; a previously described nonsense mutation (G1177X) in the alsin (*ALS2*) gene that leads to premature stop codon and may affect endosomal and vesicle transport; and finally a recurrent variant (R261H) in the *NEK1* gene, encoding NIMA related kinase-1, that has recently been associated with ALS in the Caucasian population.

Conclusion: Disease causing variants have been detected in approximately 28% (3/21) of this sporadic cohort. Our study contributed to the better understanding of the genetic background of the disorder and indicated that complex approaches are needed to understand the genetic heterogeneity of this disease.

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P10.02B
The role of 3' UTR variants in Amyotrophic Lateral Sclerosis (ALS) etiology

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of the human motor neuron system. Although the disease etiology remains unclear, ~25 genes with ALS underlying protein-coding mutations have been discovered. The mutations explain mostly familial cases, and imply genetics as an important driver of sporadic cases too. Recent studies demonstrated dysregulation of micro-RNAs (miRNAs) in ALS patients, suggesting a role of miRNAs in ALS pathogenesis. However, systematic search for disease-causing variants in miRNA genes was yet not performed. Here, we screened whole-genome sequencing data from 4281 ALS sporadic patients and 1838 controls, and explored genomic regions of 1,872 miRNAs genes and 3′ untranslated regions (3′UTRs) of 295 ALS and miRNA-related genes. The data was generated by Project MinE consortium and analyzed under collaboration. First, we developed a pipeline for functional annotation of 3'UTR and miRNA variants and called qualified variants. The

pipeline predicts loss and gain of 3′UTR miRNA binding sites and assigns miRNA variants with decreased pathogenicity score based on location in the seed, mature miRNA or precursor, respectively. Region-based rare variant association uncovered a significant association with a relevant inflammatory gene, suggesting that its tight regulation is critical. The association signal was replicated using >60,000 controls from a different cohort. This is the first report of a rare protective mutations in ALS and the first association study that implies noncoding regulation by miRNAs. The work emphasizes the ability to interrogate whole-genome sequencing for discovery of new non-coding genetic mechanisms and suggests neuroprotective immunomodulatory targets for therapy development.

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P10.03C
Investigating mechanism of *C9orf72* mutations in amyotrophic lateral sclerosis

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The GGGGCC hexanucleotide repeat expansion located in the first intron of the C9orf72 gene is the most common genetic cause for amyotrophic lateral sclerosis (ALS) as well as frontotemporal dementia (FTD). The repeat expansion is hypothesized to cause repeat-associated translation (RAN-translation), which results in toxicity by aggregation of dipeptide repeat proteins (DRPs). Though there have been many studies focusing on the toxicity of RAN-translated DPRs, the specific factors that contribute to RAN translation have not yet been identified. This study employs yeast models, which share high gene conservation with humans, to examine the mechanisms of the C9orf72 mutation in ALS. In particular, this study focused on the cloning of 149 repeat expansions of C9orf72 (C9149R). 149 repeat expansions ligated into yeast-expression vectors, p416 GAL1 and GPD, were transformed in Stbl3 E.coli and subsequently tested for stability of the 149R size. Furthermore, this study also observed stability of repeat expansions in yeast over various time periods. The sizes of 2 repeat expansions (C92R) and 40 repeat expansions (C940R) of C9orf72 in yeast were examined to observe how much shrinkage the expansions had undergone over each time period. Through the exploration of these two objectives, necessary steps were taken to further the application of C9orf72 yeast models in ALS research.

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P10.04D
Biallelic mutation in CHP1 causes human autosomal recessive ataxia by impairing NHE1 membrane targeting

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Autosomal recessive cerebellar ataxias (ARCAs) comprise a heterogeneous group of neurodegenerative disorders that affect the cerebellum, brain stem and spinal cord. Approximately 40% of the ARCA-affected patients remain genetically unresolved.

Following a combination of WES and linkage analysis, we identified a biallelic 3-bp deletion (p.K19del) in *CHP1* (Calcineurin-like EF-hand protein-1) that co-segregates with motor neuropathy, cerebellar atrophy and spastic paraparesis in two siblings of a consanguineous family. Focused screening for *CHP1* variants in two cohorts (ARCA: N=319 and NeurOmics: N=657) and GeneMatcher interrogation did not yield additional variants, thus revealing the scarcity of *CHP1* mutations. CHP1 plays a crucial role in pH regulation and ion homeostasis, by controlling the function of the Sodium/Hydrogen Exchanger-1 (NHE1, encoded by *SLC19A1*). Reduced CHP1 expression as well as NHE1 depletion cause Purkinje cell degeneration and ataxia in mouse. Moreover, loss-of-function mutation in NHE1 causes ataxia in human.

Here we demonstrate that mutant CHP1 fails to integrate into functional protein complexes and is prone to aggregation, thereby leading to diminished levels of soluble CHP1 and reduced membrane targeting of NHE1. Moreover, we show that morpholino-mediated *chp1* knockdown in zebrafish resembles the phenotype of ARCA-affected humans, leading to spastic movements, cerebellar hypoplasia and motor axon abnormalities. These defects were ameliorated by co-injection with WT, but not mutant, human *CHP1* mRNA. Collectively, our results identified *CHP1* as an ataxia-causative gene in humans, further expanding the spectrum of ARCA-associated loci, and corroborate NHE1 mistargeting as a key event underlying neuronal degeneration in the context of inherited cerebellar ataxias.

N. Mendoza Ferreira: E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; N. Mendoza-Ferreira co-holds patent 17172826.4-1401 (European patent office) for Calcineurin B homologous protein 1 inhibitors and therapeutic and non-therapeutic uses thereof.. **M. Coutelier:** None. **E. Janzen:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; E. Janzen co-holds patent 17172826.4-1401 (European patent office) for Calcineurin B homologous protein 1 inhibitors and therapeutic and non-therapeutic uses thereof. **S. Hosseinibarkooie:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; SM. Hosseinibarkooie co-holds patent 17172826.4-1401 (European patent office) for Calcineurin B homologous protein 1 inhibitors and therapeutic and non-therapeutic uses thereof.Seyyedmohsen. **H. Löhr:** None. **S. Schneider:** None. **J. Milbradt:** None. **M. Karakaya:** None. **M. Riessland:** None. **C. Pichlo:** None. **L. Torres-Benito:** None. **A. Singleton:** None. **S. Zuchner:** None. **A. Brice:** None. **A. Durr:** None. **M. Hammerschmidt:** None. **G. Stevanin:** None. **B. Wirth:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; B. Wirth co-holds patent 17172826.4-1401 (European patent office) for Calcineurin B homologous protein 1 inhibitors and therapeutic and non-therapeutic uses thereof..

P10.06B

ATP1A3 related disease: a series of new mutations expanding clinical phenotype

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Introduction: ATP1A3 gene encodes for the alpha-3 catalytic subunit of the Na+/K+ ATPase transmembrane ion pump. Gene mutations are associated with alternating hemiplegia of childhood, rapid-onset dystonia with parkinsonism or dystonia 12, cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss syndrome and catastrophic early life epilepsy.

Methods: medical charts and videos of patients were retrospectively evaluated. All patients were genetically proven for ATP1A3 gene mutations.

Results: Five patients with "atypical" phenotype were selected. Three patients were sporadic, while two were directly related. Patient one had an early-onset epileptic encephalopathy and later developed paroxysmal episodes of non-epileptic origin, monocular nystagmus and episodes of apnoea. He had a multi drug resistance epilepsy associated to developmental delay. Patients 2 and 3 represent a familial case. The proband developed at 18 months dysmetria, hypotonia and upper limbs tremor and lost the standing and sitting position. Her mother had an episode of hypotonia and seizures at the same age and after 5 years developed generalized dystonia. Patient four initially presented episodes of intermittent flaccidity and bulbar symptoms and then developed sudden onset of severe generalized dystonia, hypothonia, bradykinesia, and ataxic gait. Patient five exhibited an early onset infantile epilepsy with episodes of non-epileptic origin of hemiparesis. After several years, she abruptly developed a RDP phenotype and recently psychotic symptoms and anorexic behaviour associated.

Discussion: here we present five different cases of ATP1A3 mutations showing intermediate and overlapping phenotypes compared to the classic ones. This series expand the continuum phenotype spectrum of ATP1A3.

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P10.07C

Expanding the histopathological spectrum of CFL2-related myopathies

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Introduction: Congenital myopathy (CM) caused by mutation in cofilin-2 gene (*CFL2*) is a rare neuromuscular disorder. The few reported cases show phenotypic heterogeneity ranging from early onset and rapid progressive forms to milder myopathy characterized by slow progressive limb girdle and axial muscles weakness. Muscle histology shows features of nemaline or myofibrillar myopathy or the coexistence of both histopathological changes. We describe three new cases, from two unrelated families, of severe CM related to novel loss-of-function mutations in *CFL2*.

Materials and methods: Whole Exome Sequencing and targeted resequencing using a custom gene panel for muscular diseases were performed respectively in Patient-1 and Patient-2. Studies of muscle biopsies were performed in all patients using standard technique on quadriceps muscles.

Results: Next Generation Sequencing analysis revealed novel mutations in *CFL2:* p.(Asp86His), homozygous in Pt1, and p.(Asp79Tyr) and p.(Ser94LeufsTer6) in compound heterozygosity in Pt2 and in her older brother (Pt3). All babies presented severe neonatal muscle weakness needing continuous respiratory and nutritional support since birth. Muscle biopsies showed features consistent of nemaline myopathy with thin filament accumulations together to myofibrillar changes.

Conclusions: Muscle biopsies in our patients showed features evocative of the histopathological findings observed in $Cfl2^{-/-}$ knockout mouse model. Structural modeling analysis supports the pathogenicity of the three *CFL2* mutations and indicates that the mutated residues are involved in correct folding of cofilin-2 confirming that the activity of the protein might be important for the postnatal maintenance of sarcomeric structures. Our report expands the clinical and histopathological spectrum of *CFL2*-related myopathies.

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P10.09A

A report of a family of intermediate Charcot-Marie-Tooth disease with concomitant mutations in the *GNB4* and *DNM2* genes

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Charcot-Marie-Tooth disease (CMT) is the most common inherited neuromuscular disorder with an estimated prevalence of 1 in 2500 people. Hallmarks include symmetric foot deformities, slowly progressive weakness and wasting in the distal parts of upper and lower limbs, and length-dependent sensory loss. Positive symptoms such as paraesthesias and pain may occur. Hereditary neuropathies are ideal candidates for diagnosis by Next-generation sequencing (NGS) approaches because similar phenotype is caused by variants of different genes. We report the unusual occurrence of CMT, caused by mutations c.847C>T (p.Arg283Cys) in *GNB4* and c.1609G>A (p.Gly537Ser) in *DNM2*, in a dominant geneaology. The phenotype of all affected family members was rather uniform with symptoms starting in the lower limbs. Initial signs were foot deformity and gait abnormalities in early childhood. Nevertheless severity of progressive distal weakness of upper limbs, postural tremor and hand deformities were different in some of the affected family members. The digenic effect of two pathogenic variants in different genes may modulate the phenotype, since both gene products are involved in endosomal sorting and cell signalling, possibly interacting in similar pathways. This study and previously reported cases of CMT caused by concomitant gene alterations is a notice to look for several causative mutations in families having phenotypic variability or unusual expression.

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P10.11C

Comprehensive copy number variant profiling in 234 diagnosis-resistant myopathic patients

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Next generation sequencing (NGS) has led to an increase in the diagnosis of non-specific skeletal muscle disorders with a detection rate of single nucleotide variants or small ins/dels of 40%-60%. Mutations in unknown genes, multifactorial or polygenic conditions, or elusive variants such as deep intronic mutations, variants in regulatory elements, trinucleotide repeat expansions or copy number variants (CNVs) may occur in the patients remaining undiagnosed. An extensive NGS study of 504 genetically undetermined patients with clinical diagnosis of muscular dystrophies, congenital myopathies or other conditions affecting muscles was performed, identifying putative causative mutations in 218/504 cases. We recruited 234/286 unsolved patients to study the impact of CNVs in skeletal muscle disorders and potentially to improve the diagnostic rate. All patients were analyzed by Motor Chip, a custom CGH-array to identify deletions and duplications in neuromuscular disorders. We found non-polymorphic CNVs in 22 patients (9.4%). In 12 patients (5.1%), the identified CNVs were considered responsible for the observed phenotype. Other 10 patients (4.3%) had CNVs of uncertain significance (VUS). Although these VUS may not act as primary disease drivers, we cannot exclude that some of them may act as modifiers, contributing to the observed phenotype. Our study has allowed the genetic diagnosis in unsolved patients and identification of previously undescribed rearrangements in muscle genes. It confirms that deletions and duplications account for 5-10% of patients affected by a skeletal muscle disorder without a molecular diagnosis, explaining a not exiguous number of unsolved cases.

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P10.12D

Crisponi / Cold-induced sweating syndrome: Seven new cases and two novel mutations

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Introduction: Sohar-Crisponi Syndrome with the other name cold induced sweating syndrome was first recognized by Sohar in 1978 and then renamed in 1996 by Crisponi. Today it is defined as an autosomal recessive disorder characterized by muscular spasms of facial muscles, tetany, difficulty in swallowing and excess salivation, attacks of fever during first two years of life. During childhood tetany and fever attacks ceases but cold induced sweating and thermodysregulation follows. In most patients typical facial features, camptodactyly with tapering fingers and kyphoscoliosis are also present. Most of the patients have mutations in CRLF1 gene, in recent years two additional genes are defined: CLCF1 and KLHL7.

Materials and Methods: We collected the datae of 6 families with 7 affected members. All were evaluated for neurological, metabolic and ophthalmologic pathologies. CRLF1 gene analysis was done in all families.

Results: Four of the patients were diagnosed during first months of life, two were one month old, the oldest one was 10 years old during diagnosis. All have typical face, camptodactyly, abnormal finger and hand morphology and difficulty in swallowing. Fever attacks were present in infants. Thermodysregulation, and hyperhydrosis was present in older patients. All the patients had CRLF1 mutations. Two of the mutations were novel.

Conclusion: As new cases as our series with novel and known mutations are described, more information will be available about this syndrome and this will help clinicians to diagnose and follow the patients more efficiently.

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P10.13A

Deletion & duplication mutations in Duchenne muscular dystrophy in southern Iranian children

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Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked disorders caused by mutation within the dystrophin gene. Our study has identified 28 Iranian families collected from Narges Genetic Lab, southwest of Iran, Ahvaz. All cases were subjected to

complete clinical evaluation pedigree analysis, electromyography studies, estimation of serum creatin phosphokinase (CPK) level and DNA analysis. Sample's DNA was analyzed by multiplex ligation-dependent probe amplification (MLPA). In this study deletion rate was 75% (21/28) and was more frequent in the distal end exons whereas duplication rate was 25% (7/28) and it was more frequent at proximal exons. Majority of the deletion (11/21, 52%) were located on the distal hot spot region that encompasses exons 45-55 and 33% of the deletion (7/21) were located at the proximal hot spot region (exon 1-16). Majority of exon duplication 71.5% (5/7) located at the proximal hot spot region (exon 1-16) and 28.5% (2/7) located at the distal hot spot region (exon 50-62). Single exon deletions were present in 8/28 families (28.5%) with the most common in exon 45 (2/21, 9.5%). One family had a de novo single exon deletion (exon 51) and whole dystrophin gene was deleted in only one family. This finding indicate that as with deletions, duplications occur nonrandomly but with a dramatically different distribution. Duplication frequency is highest near the 5' end of the gene.

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P10.14B

DMD noncontiguous duplications in duplication-normal-inversed duplication (Dup-Nml-Dup/inv) manner revealed by targeted sequencing

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Introduction: Complex Genomic Rearrangements(CGRs) have been demonstrated in DMD. However, few CGRs in DMD have been described at the nucleotide level thus difficult to predict the consequence of the CGRs.

Materials and methods: Case1, a DMD patient, harbors duplications of exon53-55 and exon57-79 in DMD gene. Case2 is a female with growth retardation and craniosynostosis. SNP array identified a duplication fragment included DMD gene. MLPA revealed the patient had duplications of Dp427c, exon1-2 and exon5-7. They were sequenced by whole DMD target sequencing. Breakpoints of the CGRs were detected by split-read method to reconstruct the structural changes of the gene and to investigate the mechanism of the CGRs.

Results: Both cases shared the same duplication event (Dup-Nml-Dup/inv). In case1, a fragment comprised of exon57-79 and two upstream genes(FTHL17, TAB3) in an inverted orientation and exon53-55 is inserted in intron55. The breakpoint is in intron2 in case2. The insertion is an inverted duplication of exon5-7 followed by a duplication of exon1-2 started further upstream of the DMD gene. Thus, their DMD reading-frame would be disrupted. Both CGRs involved microhomology and small insertions at the breakpoints. In Case1, SNP sequencing results indicated that the de novo duplication mutation arose in the allele that originated from the grandfather.

Conclusions: This study has report a method to uncover CGRs in nucleotide level and revealed a novel type of DMD CGR. Knowing the genetic alteration help to predict the consequence of the CGRs and provides insight into the molecular basis of this genomic rearrangement.

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P10.15C

Duchenne muscular dystrophy: gene therapies in low-income counties

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Duchenne muscular dystrophy (DMD; OMIM ref. 310200) is an X-linked disease that affects 1 in 3600-6000 live male births. DMD occurs as a result of mutations in the dystrophin gene (Xp21.2). Mutations cause a dysfunction in, or lack of, protein dystrophin that is essential for muscle cell stability. Milder allelic forms of the disease are intermediate muscular dystrophy and Becker muscular dystrophy. This is a descriptive study of 87 patients diagnosed with DMD at the CMG of Armenia from 2011 to 2017. Patients were reviewed by neurologist and a clinical geneticist; the clinical diagnosis was established and followed by other supportive investigations. A diagnosis of DMD was confirmed by molecular testing using multiplex PCR (in Armenia) and *dmd* gene sequencing (in France). From 2012 the management of DMD patients in Armenia is done according to DMD Care Considerations Working Group's recommendations. Here we presented the spectrum of DMD mutations which were observed among 87 patients with muscular dystrophies in Armenia. 48% of these cases are with confirmed DMD mutations, mainly deletion(s), as well as duplication(s) and point mutations. Our available data suggest that up to 40% of DMD patients have genotypes amenable to exon skipping or Ataluren therapies. Many promising therapeutic strategies have since been developed, however availability of those therapies is restricted for low-income counties such as Armenia. Having no access to advanced therapies has fatal consequences for the patients,

as well as for the society, forcing affected families to seek help outside of their country.

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P10.16D

Identifying and counseling patients amenable to mutation-specific therapies in Duchenne muscular dystrophy: knowledge of resources will fuel genetic counselors' impact

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Introduction: Mutation-specific therapies for Duchenne muscular dystrophy (DMD) are in clinical trials worldwide. More than 60% of DMD patients have *DMD* gene mutations amenable to two current approaches: stop codon read-through and single exon skipping.

Methods: We identified gaps in DMD genotyping and examined potential solutions for genetic counselors to help overcome barriers in determining patient eligibility for mutation-specific therapies.

Results: Access to genetic testing is one barrier. A survey of 27 physicians who managed >2,000 DMD patients showed substantial variability in genotyped patients (≈25%-100%). In Europe, multiplex ligation-dependent probe amplification is widely adopted, while availability of sequencing procedures is nonhomogenous, especially in Eastern countries. Awareness of financially viable genetic testing options is essential (eg, International DMD, University of Ferrara [Europe]; Decode Duchenne [USA, Canada]. Accurate, unequivocal mutation interpretation presents a second barrier. While nonsense mutations are amenable to stop codon read-through, other *DMD* deletion mutations vary in amenability to exon skipping, depending on the exons involved. To direct patients to appropriate therapy, clinicians must discern if a mutation is appropriate for exon skipping and amenable to a particular exon-skipping therapeutic. This pinpoints the importance of correct genotyping and genetic counselor involvement in mutation interpretation.

Conclusions: Access to interpretation tools and guidance is paramount for genetic counselors to advise both patients and providers. We will review the *DMD* gene exon map, Leiden DMD database, and Decode Duchenne mutation-specific resource as tools to help genetic counselors determine DMD patients' eligibility for exon skipping trials, with the goal of increasing genotyped patients.

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P10.17A

Exome sequencing identifies 2 novel disease-causing DYNC1H1 mutations in patients with spinal muscular atrophy or mental retardation

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Mutations in DYNC1H1 gene cause several autosomal dominant diseases including type 20 Charcot-Marie-Tooth, type 13 mental retardation and lower extremity-predominant spinal muscular atrophy 1. These diseases have several common clinical symptoms. DYNC1H1 gene encodes a large (over 530 kD) crucial subunit of cytoplasmic dynein complex, a cytoskeleton motor.

We did exome sequencing of 4 blood samples using Agilent FocusedExome enrichment system and Illumina HiSeq2500. For variant calling and pathogenicity scoring we followed ACMG guidelines. We also confirmed disease-causing mutations by Sanger sequencing.

Of 4 studied patients one had symptoms of spinal muscular atrophy and 3 others had mental retardation with seizures and polymicrogyria.

We identified heterozygous mutations in DYNC1H1 gene. Two variants (c.9749_9751delAAG and c.2029G>A) were previously described as uncertain significance alleles. Two others (c.751C>T and c.5882A>T) were not listed in dbNSFP, Clinvar, OMIM, HGMD, 1000Genomes and ExAC databases.

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P10.19C

Whole-genome sequencing detects a large genomic inversion disrupting the *DMD* gene in a Becker muscular dystrophy patient

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Introduction: Duchenne and Becker muscular dystrophies (D/BMD) are caused by pathogenic variants in the dystrophin (*DMD*) gene. Being the largest *locus* of the human genome (2.3Mb, Xp21.1), *DMD* is particularly prone to genomic rearrangements, often intragenic multi-exonic deletions or duplications (~70% of cases). Single nucleotide variants are identified in most of the remaining D/BMD cases, whereas complex genomic rearrangements encompassing *DMD* are rarer.

Materials and Methods: Routine techniques failed to detect pathogenic *DMD* variants in a BMD patient with progressive muscle weakness, mild intellectual disability and dystrophic muscle showing irregular dystrophin labelling. *DMD* transcript analysis (RT-PCR and cDNA-MLPA) was performed, as well as low-coverage whole-genome sequencing (WGS) using the 10xGenomics Chromium system.

Results: RNA analysis showed the absence of exons 75-79. While automated structural variant calling from WGS was inconclusive, visual BAM file inspection showed a possible breakpoint within intron 74 of *DMD*. Some reads had homology with a region located upstream of the *PRDX4* gene (Xp22.11). Similarly, some reads in that location showed homology with inverted intron 74 *DMD* sequences. Breakpoint PCR and Sanger sequencing confirmed the presence of a ~8Mb inversion. Abnormal *DMD* transcripts were subsequently identified, some of which contained segments from the region upstream of *PRDX4*.

Conclusions: The patient's phenotype is explainable by this *DMD* inversion with concomitant loss of the C-terminal region of dystrophin. Besides expanding the *DMD* mutational spectrum, this report reinforces the importance of WGS in clinical genetics, having the potential to detect a wide variety of mutation types.

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P10.20D

A non-pathogenic duplication of *DMD* exon 45-51, inserted in chromosome 17, in three Danish patients

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Introduction: Dystrophinopathies are caused by pathogenic variants in the DMD-gene. Whole exon deletions or duplications account for the majority of cases of DMD-related disease (~65%). When these variants are found it is important to investigate whether they alter the reading frame, and special care should be taken when found incidentally or prenatally since not all DMD-duplications or deletions cause disease.

Materials and method: Blood and chorionic villus samples were analyzed using chromosome microarrays and FISH.

Results: We report three cases with incidental finding of an intragenic duplication encompassing exon 45-51 (out of 79 exons) in the DMD-gene by chromosome microarray analysis. Case 1 is an asymptomatic 44-year-old male, investigated as a parental control to an amniotic sample. Case 2 is a male fetus referred because of increased risk of trisomy 21 at first trimester screen. The duplication was not inherited from the mother. Unfortunately, the father was unavailable for study. Case 3 is also a male fetus referred because of increased risk of trisomy 18 at first trimester screen. The duplication was inherited from a healthy mother. The duplicated material was in all cases inserted in chromosome 17q.

Conclusion: DMD duplications are a known cause of DMD-related disease. However, caution should be made when found incidentally and prenatally, and assumptions regarding disease prediction should not be made without further investigations. This is especially important regarding duplications since they can be inserted in other chromosomes and thus do not affect the DMD-reading frame.

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P10.21A

Molecular analysis of an argentine dystrophinopathy cohort: diagnostic algorithm, genetic assessment and *DMD* gene characterization

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Introduction: Dystrophinopathies are X-linked recessive diseases caused by mutations in *DMD* gene. Hitherto there is no effective treatment for these pathologies, which enhances the importance of performing genetic assessment in order to detect mutation carriers and prevent diseased newborns. However, two mutation-specific gene therapies were recently approved: Exon 51 Skipping (Eteplirsen) and Premature Stop Codon Read-through (Ataluren). Therefore, accurate detection and characterization of the causing mutation is essential to allow genetic counseling, patient follow-up and determine the suitable gene therapy.

Materials and methods: We have analyzed 200 boys with clinical diagnosis of Dystrophinopathy, 12 symptomatic women, 240 females at-risk of being carriers and 15 prenatal diagnoses. A diagnostic algorithm was designed for each case, implementing MLPA, PCR, Whole Exome Sequencing, Sanger Sequencing, STRs segregation analysis and HUMARA assay.

Results: The selected strategy allowed disease confirmation in 71.7% (152/212) of the affected boys and symptomatic females. 12 were candidates for Eteplirsen, while 22 were suitable for Ataluren. On the other hand, we were able to establish as carriers 72/255 women/fetuses, while could exclude from being carriers/affected 143/255. As for gene characterization, we could establish an association between the most frequent deletion/duplication intron breakpoints and the abundance of STR loci and, we have detected 3 haplotypes blocks within the SNPs identified by the Exome technique.

Conclusions: In the present work, we have characterized a Dystrophinopathy argentine population and contributed to the understanding of the genetic/molecular basis of these pathologies

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P10.22B

***ECEL1*-related distal arthrogryposis: The tale of the tongue**

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Introduction: Distal arthrogryposis (DA) a an important subgroup of arthrogryposis multiplex congenita, characterized by congenital joint limitations with involvement of especially, but not exclusively, the distal extremities. It is a heterogeneous condition. However, in many DA patients, the genetic defect remains to be elucidated. Recently, mutations in *ECEL1* were identified as a novel cause of autosomal recessive distal arthrogryposis.

Materials and methods: We report on a girl with congenital DA with flexed fingers and adducted thumbs, limited flexion of the knees, abnormal position of the toes and vertical grooves on both shins. Already at birth, a striking central atrophy of the tongue was noted. In addition, she had a right-sided ptosis and limited facial expression. Radiographs demonstrated dysplastic acetabulae and a right-sided hip luxation. Brain MRI was normal. Family history was unremarkable, apart from parental consanguinity.

Results: Sanger sequencing of *ECEL1*, located in a 24 Mb homozygous region, demonstrated the homozygous c.2023G>A, p. Ala675Thr variant affecting a highly conserved amino acid and previously associated with distal arthrogryposis. Discussion: Our case demonstrates the classic *ECEL1* phenotype. Besides DA, the central atrophy of the tongue is present in the majority of cases. Congenital ptosis and limited facial expression are also frequent findings. Previously unreported, but remarkable findings in our patient were the vertical grooves on the shins. Long term complications may include scoliosis and hyperlordosis, as well as restrictive respiratory insufficiency. We demonstrate that the *ECEL1*-phenotype is clinically recognizable enabling single gene analysis for the diagnosis of this specific DA subtype.

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P10.23C

Phenotypic significance of variants in the *TTR* promoter: Familial Amyloid Polyneuropathy (TTR-FAP) onset

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Introduction: Val30Met variant in transthyretin gene (*TTR*) is causative for Familial Amyloid Polyneuropathy (FAP). Substantial phenotypic heterogeneity has been described in patients with Val30Met, including in age-at-onset. Consequently, other variants in *TTR* locus, beyond the TTR-FAP causing variant, could play a regulatory role in its expression level and modify disease expressivity. We aim to identify genetic modifiers of disease onset that may contribute to this clinical variability.

Materials & Methods: We genotyped the promoter and coding and flanking regions of *TTR* gene in Val30Met TTR carriers. An intensive in silico analysis was performed in order to understand a possible regulation of gene expression.

Results: We identified 12 known variants in the promoter region and 2 known intronic (rs36204272, rs1791228), one 3' UTR (rs62093482) and 2 known exonic variants (rs28933981 and rs1800458). Importantly, our analysis revealed variants significantly associated with age-at-onset, one of which is a CA repeat (rs71383038) in promotor region. In addition, unreported and very interesting results in the in silico analysis were found since we observed some alterations in the mechanism of splicing, transcription factors binding and miRNAs binding.

Conclusions: Our findings raise an interesting possibility that TTR variants could be genetic modifiers in Val30Met carriers and might influence disease variability. Furthermore, our findings suggest that variants within promoter region can modify disease expressivity and have the potential to be a biomarker to identify disease onset within asymptomatic carriers at risk of developing TTR-FAP promoting a better follow-up of Val30Met carriers.

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P10.24D

A new form of fetal akinesia syndrome is due to mutations in the SLC5A7 gene

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Introduction: Severe fetal akinesia results in a recognizable deformation sequence with variable pre- and postnatal phenotype including: polyhydramnios, reduced spontaneous movements, arthrogryposis, and pulmonary hypoplasia. The primary causes are genetic, heterogeneous, and due to defects of the motor pathway. A subgroup of these conditions is characterized by endplate specific mutations of the neuromuscular junction. Recently, recessive mutations in the SLC5A7 protein coding for the high affinity choline transporter CHT1 have been related to a continuum of phenotypes characterized by congenital myasthenia and episodic apnea (Bauché et al., 2016, Wang et al., 2017). We report the independent identification and characterization of a new family with a lethal form of disease due to a novel homozygous mutation in SLC5A7 and review the two previously published families with a similar phenotype proposing a genotype-phenotype correlation for a new subclass of lethal fetal akinesia.

Materials and methods: A detailed clinical description of a new consanguineous family with two affected children with fetal akinesia is provided as well as *in vitro* functional characterization of the novel CHT1 mutation including a rescue experiment.

Results: While SLC5A7 cell-surface biotinylation showed that the mutant is able to reach the plasma membrane, no choline transport was detected. Functional rescue of the mutant with different chemical chaperones failed to compensate the altered function.

Conclusions: This study brings further clinical and functional evidence for a novel pathogenic mutation in CHT1, and proposes that recessive mutations of the intracytoplasmic protein domains of SLC5A7 are responsible for a lethal form of fetal akinesia.

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P10.25A

Whole exome sequencing in floppy child syndrome patients with a particular consideration of neuromuscular disorders

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Introduction: The„floppy child syndrome" is one of the most unambiguous medical condition presenting at birth or in early infancy. We aimed to assess the etiology of this condition with focus on neuromuscular symptoms.

Material and methods: Till now, 120 probands presenting generalized hypotonia at birth or in neonatal period with excluded major genetic causes (e.g. SMA), were included in the study. For 75 of them, the exome sequencing (WES) was performed using QXT Sure Select Human All Exome v.6. In selected cases, directed analysis using classic Sanger sequencing / MLPA was performed.

Results: Directed analysis revealed the presence of pathogenic variants in *MTM1* in three patients with myotubular myopathy and in *ACTA1* in four patients with nemaline myopathy. First analysis of WES data included known genes related to neuromuscular disorders. The most common mutated genes were: *LMNA* (4 patients with *de novo* variants) and *RYR1* (5 patients, 2 compound heterozygotes, 3 heterozygotes). In single patients, potentially pathogenic variants in *LAMA2*, *PIEZO2*, *SEPN1*, *PMP22*, *SGCA*, *PIGA*, *DNM2*, *PPP2R1A*, *COL12A1* or *COL6A1* genes were found, analyzed for the inheritance in families and confirmed to be related to the patient specific phenotype.

Conclusion: The genetic cause of floppy child syndrome was found in 27/120 (22.5%) patients. The first-line analysis for the presence of mutations in known neuromuscular genes seems to be a good starting point for further studies of new genes related to the etiology of "floppy child syndrome".

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P10.26B

Targeted methyl-Seq quantification by NGS technology for routine diagnostic of FSHD

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Facioscapulohumeral Muscular Dystrophy (FSHD) is associated to hypomethylation of the D4Z4 microsatellite, leading to an increased expression of the DUX4 gene which is proposed to cause progressive muscle atrophy and ultimately FSHD pathology. Chromatin changes are caused by contractions to less than 11 units of the D4Z4 repeat (FSHD1) or by pathogenic variants in chromatin regulatory proteins (FSHD2). We developed a novel NGS-based method that allows us to diagnose FSHD and distinguish FSHD1 from FSHD2 in a single approach. Enrichment of 4qA (A) and 4qAL (AL) associated D4Z4 repeats as well as all 4q35 D4Z4 repeat units (DUX4) was performed using nested PCRs after bisulfite conversion of genomic DNA (EpiTect). DNA libraries were prepared with the NEBNext Ultra DNA Library Prep Kit and sequenced on an Illumina MiSeq System. Reads were mapped to A and AL using bwameth. Mean methylation levels were calculated with an in-house script. We analyzed 21 patients with a confirmed FSHD diagnosis and 21 negative controls. We could reliably confirm the hypomethylation status in all patients. Furthermore, the results show a correlation of methylation levels to the disease status. In addition, we could distinguish FSHD1 from FSHD2 by DUX4 methylation levels, as due to a chromatin dysregulation DUX4 is hypomethylated in FSHD2 as well. In conclusion, sensitive detection of methylation levels in the D4Z4 array can give insight into the clinical progression levels more exactly than currently

available methods. Additionally, a much higher yield and throughput in FSHD diagnostics can be achieved.

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P10.27C

Whole-exome sequencing is effective for clarification of unsolved causes of hereditary neuropathies in Czech patients

L. Sedlackova[1], P. Lassuthova[1], J. Neupauerova[1], D. Safka Brozkova[1], D. Stanek[1], J. Haberlova[2], R. Mazanec[3], P. Seeman[1]

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Introduction: Inherited peripheral neuropathies (IPN) are the most common monogenic neurological disorder. It is a clinically and genetically extremely heterogeneous group with more than 90 genes already involved. Whole-exome sequencing (WES) was used in patients previously unsolved using standard diagnostic methods and clinical experience.

Patients and Methods: Here we present WES results of 47 undiagnosed patients with IPN from 38 unrelated families tested previously in our diagnostic lab for better known IPN types and genes.

Results: Causative mutations were found in thirteen patients (34%) and in twelve genes to date. In six patients we detected mutations in known IPN genes (*SOD1*, *MFN2*, *BSCL2*, *DNM2*, *SMN1*, *AIFM1*) and in four of them the found mutation could confirm previous just novel findings (*MORC2*, *SLC25A45*, *DRP2*, *GNB4*). But we also revealed three novel causative gene-IPN associations in frame of international collaboration (*ATP1A1*, *HARS*). We uncovered mutations even in well-known genes previously tested in single gene tests, which highlights that WES may detect also variants that have been missed by Sanger sequencing during routine diagnostics. Some of the causative mutations were identified immediately after publication of their causality by the WES data reevaluation.

Conclusion: WES represents efficient tool for clarification of rare, novel or unusual IPN types and it has an important potential for novel candidate genes in familiar cases. In unsolved patients WES data should be re-evaluated every 6 months due to the continual increase of novel published genes and variants. Supported by: MH CR AZV 16-30206

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P10.28D

Diagnostic utility of clinical exome sequencing in a cohort of patients with hereditary polyneuropathies

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Normal 0 false false false EN-GB X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-ansi-language:EN-GB;} **Introduction:** We describe clinical characteristics in a cohort of patients with hereditary polyneuropathies (N=50) and compare them to the results of clinical exome sequencing (CES). **Methods:** A cohort was devided in two clinical groups. First group with positive CES findings (N=21) and second with negative CES findings or variants of unknown significance (N=29). Clinical and genetic characteristics were analyzed. **Results:** In the first group almost half of the patients (N=10) had clinical presentation of Charcot-Marie-Tooth polyneuropathy with positive electromyography findings, slowly progressive clinical course with moderate severity of disease, with a beginning as children or as young adults and positive family history. Four patients with Charcot-Marie-Tooth polyneuropathy were sporadic cases, four patients had additional central nervous system findings and three patients were found to have myopathy based on molecular results. Negative CES findings were mostly (N=13) correlated to sporadic cases and electromyography findings of axonal polyneuropathy. **Conclusions:** Clinical exome sequencing was found to be conclusive specially in hereditary polyneuroptahies with Charcot-Marie-Tooth clinical presentation and positive family history. Yield of CES in cases of hereditary polyneuropathy was 42%. Sporadic cases with electromyography findings of axonal polyneuropathy were often found negative. <!--EndFragment-->

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P10.29A

Spectrum of genetic causes of hereditary spastic paraplegias among Czech NON-SPG4 patients

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Introduction: Hereditary spastic paraplegias (HSP) are clinically and genetically heterogeneous disorders of the central motoneuron. Typical clinical feature is progressive bilateral spasticity and weakness of the lower limbs. All types of inheritance and causative variants in already more than 70 genes hase been described in HSP. The most common cause are pathogenic variants in the *SPAST* (SPG4) gene.

Materials and methods: We aimed to map the genetic spectrum of HSPs in 72 Czech non-SPG4 uncomplicated HSP patients. Fourty-three patients were with positive family history and 29 were sporadic.Targeted Enrichment of all coding exons of 38 genes associated with uncomplicated HSP with our probe design was used for MPS.

Results: Causal variants were found in 16 patients among the group of 72 HSP patients (22.2%). Slightly more patients were detected in the group of familial patients (10/43; 23.3%) vs. sporadic patients (6/29; 20.1%). Causative variants were found in *SPG11* (4 x), *REEP1*/SPG31 (3 x), *KIF5A*/SPG10 (2 x), *SPG7* (2 x), *NIPA1*/SPG6, *CYP7B1*/SPG5, *KIAA0196*/SPG8, *FA2H*/SPG35 and *SPAST* (all 1x). Surprisingly ATL1 variant was found only once and it finaly turned to not causal because of presence in the healthy parent.

Conclusion: SPG11 seems to be the most common type of HSP among Czech non-SPG patients. SPG3A and SPG7 seem not to be so frequent then in other populations. Together nine genetic types of HSP were found among 72 Czech uncomplicated non-SPG4 HSP patients. Project supported by: Ministry of Health of the Czech Republic grant nr.15-31899A.

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P10.31C

The impact of next generation sequencing on the diagnosis of pediatric-onset hereditary spastic paraplegias: new genotype-phenotype correlations for rare HSP-related genes

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Background: Hereditary spastic paraplegias (HSP) are clinical and genetic heterogeneous diseases with more than eighty disease genes identified thus far. Studies on large cohorts of HSP patients showed that, by means of current technologies, the percentage of genetically solved cases is close to 50%. Notably, the percentage of molecularly confirmed diagnoses decreases significantly in sporadic patients.

Objectives: To describe our diagnostic molecular genetic approach on patients with pediatric-onset pure and complex HSP.

Methods: Forty-seven subjects with HSP from 44 unrelated families underwent molecular screening of 113 known and candidate disease genes by targeted capture and massively parallel sequencing. Negative cases were successively analyzed by multiplex ligation-dependent probe amplification (MLPA) analysis for the *SPAST* gene, and high-resolution SNP array analysis for genome-wide CNV detection.

Results: Diagnosis was molecularly confirmed in 29 out of 47 (62%) of patients, most of whom had clinical diagnosis of cHSP. Although *SPG11* and *SPG4* remain the most frequent cause of respectively complex and pure HSP, a large number of pathogenic variants were disclosed in *POLR3A*, *FA2H, DDHD2, GLUT1, ENTPD1, ERLIN2, CAPN1, ALS2, ADAR1, RNASEH2B, TUBB4A, ATL1* and *KIF1A*. In a subset of these disease genes, phenotypic expansion and novel genotype-phenotype correlations were recognized. Notably, SNP array analysis did not provide any significant contribution in increasing the diagnostic yield.

Conclusion: Our findings document the high diagnostic yield of targeted sequencing for patients with pediatric-onset, complex and pure HSP. MLPA for *SPAST* and SNP array should be limited to properly selected cases based on clinical suspicion.

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P10.32D

***HINT1* gene mutations are frequent cause of CMT in Russia**

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Introduction: Inherited peripheral neuropathies (IPNs) are neuromuscular and neurodegenerative disorders that affect the peripheral nervous system (PNS). More than 100 different subtypes have been identified. *HINT1* mutations were revealed as the reason of IPNs and Charcot-Marie-Tooth neuropathy (CMT).

Materials and Methods: Massive parallel sequencing (MPS), multiplex ligation depended probe amplification (MLPA) and Sanger's sequencing have been used. We investigated 700 patients without mutation in genes of frequent CMT from 2000 CMT patients. Also 1000 uninspected unrelated persons were tested.

Results: In four of five Russian patients with neuromiotonia and axonal neuropathy the homozygous c.110G>C (p. Arg37Pro) *HINT1* mutation were identified. Thirty CMT patients from 700 with c.110G>C homozygous mutation have been revealed. Two compound-heterozygotes c.110G>C mutation with earlier not described mutations (c.112-1delG and c.281A>G (p.Tyr94Cys)) were detected. Three heterozygotes of c.110G>C mutation in *HINT1* have been revealed during the research. We consider that CMT phenotype of these patients isn't caused by *HINT1* mutation and they are carriers of c.110G>C mutation. The c.110G>C *HINT1* mutation was revealed at 4 of 1000 persons. Therefore the carriage of c.110G>C mutation in the Russia is 1 of 250 persons. Thus *HINT1* mutations are not less than 1,6% of all CMT types in Russia.

Conclusions: For the first time in Russia the research of *HINT1* gene mutations at CMT is conducted. The unique c.110G>C frequency data of heterozygotic carriages in Russia are obtained. Two new mutations in *HINT1* gene were described.

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P10.35C

An extension to phenotypic spectrum of ATP1A2 gene or an unsolved case?

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Background: Mutations in ATP1A2 gene have been associated with autosomal dominant familial hemiplegic migraine type 2 syndrome. We aim to present an atypical presentation of a published pathogenic variant in the ATP1A2 gene, in a patient that does not show the classic episodic attacks of hemiplegia, oculomotor and autonomic disturbances, nor the progressive cognitive impairment.

Method: The patient was referred from the neurology clinic for diagnosis and genetic counselling. Clinical assessment and whole exome sequencing(trio) was performed.

Results: The 15 years old male patient had a history of failure to thrive, gastroesophageal reflux since age 2 months, delay in motor development(sits at age 1 year, walks at 4 years), choreoathetotic movements, difficulty in walking and dystonia, without seizures. He has normal hearing, but difficulty in speech. Brain MRI, performed at different times(age 5,10,13 and 15 years) did not show abnormalities. Brain spectroscopy performed at age 15 years, was unremarkable. Normal nerve speed conduction and EEG were repeatedly normal. Parents are healthy not consanguineous, without significant family history. Whole exome sequencing revealed a heterozygous variant c.1816G>A p.(Ala606Thr) in the ATP1A2 gene, segregation study concludes de novo occurrence. No other variant relevant for the phenotype was detected. The evolution of our patient was progressive not episodic as described gene associated diseases.

Conclusions: Although there is a significant candidate variant in the ATP1A2 gene the phenotype is discordant. Questions that remain: Are they linked? Is this a new pathogenic implication of the ATP1A2 gene?

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P10.36D

New method for detecting mtDNA deletions from massively parallel sequencing data

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Introduction: Human mitochondrial genome (mtDNA) is a circular DNA of 16 Mb in size. Mutations can cause several

inherited diseases, many of which are neuromuscular. The mutational rate compared to nuclear DNA is very high. However, because every cell contains several copies of mtDNA, mutations are heteroplasmic (0-100%). All mtDNA mutations have to exceed a threshold on the level of heteroplasmy to have an effect on the cell. Previously used methods for detecting mtDNA deletions (real-time PCRs and Southern blot) do not define the breakpoints accurately and the heteroplasmic rate is a rough estimation.

Materials and methods: We have analyzed 60 samples for mtDNA deletions. DNA was isolated from muscle. Samples included controls harboring a single mtDNA deletion (n = 2) and multiple deletions (n = 4). Amplification of the mtDNA was performed using long-range PCR followed by massively parallel sequencing. Analysis of mtDNA deletions was done with a new algorithm generated for detecting both single and multiple mtDNA deletions. Results of the samples were also compared to results from MLPA analysis.

Results: Using this method we were able to detect single and multiple deletions and also deletions that are within each other. In addition, the exact breakpoints are disclosed and the heteroplasmic rate defined. Based on the analysis of the control samples, the new method seems to be reliable in detecting all types of mtDNA deletions.

Conclusions: Our new detection method sets the mtDNA analysis to a new level and increases understanding of the mtDNA deletions' variety and expectantly their clinical relevance.

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P10.37A

Cumulative effect of rare functional variants in Multiple Sclerosis associated genes

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Genome Wide Association Studies identified over 100 Multiple Sclerosis (MS) risk loci. However, these studies are usually focused on common variants, mainly located in non-coding sequences. We aimed at searching for rare functional variants in MS associated loci and assessing if the genes in these regions show an imbalance of rare variant frequencies (burden) between MS patients and healthy controls (HC). We sequenced the coding and UTR regions of 100 MS genes in 588 Italian MS and 408 matched HC, pooled in groups of 12 individuals using an approach implemented by our group (Anand 2016). The burden of rare functional variations in MS susceptibility was assessed with 3 gene-based statistical tests (Weighted-Sum Statistic, C-alpha test, Fisher hybrid test). Variants were selected using various filtering criteria based on allelic frequency, functional impact according to in-silico prediction and functional annotation of regulatory regions. Seventeen genes showed a statistically significant burden and were thus sequenced in an independent cohort of 504 MS and 504 controls, using the same pipeline developed for the discovery phase. After meta-analysis of the two cohorts, we observed a significant burden ($p \leq 0.039$) for three genes, each in a different MS region, with at least one filter and one burden test. In conclusion, this work suggests that the association signal in MS loci may be driven by a burden of rare variants in a single gene and confirms the role of rare variants in the susceptibility to autoimmune diseases, particularly to MS. Supporting grant: FISM 2015

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P10.40D

NGS-testing using an expanded gene panel on Neuromuscular patients in Norway-results and limitations

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Introduction: Neuromuscular disorders (NMDs) contain a broad group of disorders often with overlapping clinical signs difficult to differentiate. Some of the more frequent

diseases such as Limb-Girdle muscular dystrophy and Charcot-Marie-Tooth are getting increasingly heterogeneous as genetic discoveries proliferate and different genetic testing strategies evolve. Through a 2-year period, we have offered an expanded gene panel based on Next-generation Sequencing (NGS) of patients with NMDs. We performed NGS-analysis with a gene list starting with 256 genes that were expanded to 328 genes during the study on 200 patients where standard testing had yielded no specific diagnosis.

Methods and Results: NGS was performed using Illumina TruSight One Sequencing panel (4813 genes) and run on an Illumina NextSeq 500 Desktop Sequencer. Analyses were done using Illumina BaseSpace BWA Enrichment Workflow and annotation, filtration and variant curation were done using Cartagenia Bench and Alamut Vision. Among 200 patients, we received a diagnosis in approximately 40% of the patients, including some patients with variants considered to be of uncertain clinical significance (VUS). Many of the patients presented with atypical phenotypes in relation to the genetic diagnosis. Low coverage on exon 1 is a common problem, and detection of rearrangements is a limitation of the method, particularly in NMD patients.

Conclusions: An extended gene panel designed for NMDs resulted in a high diagnostic rate similar to whole exom sequencing studies (WES) on NMD patients. We recommend using a broad gene panel for NMDs as phenotypic overlap is getting more evident from recent genetic findings.

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P10.41A

Evaluation of NIPA1 repeat as potential disease modifier in Italian amyotrophic lateral sclerosis cases carrying C9ORF72 expansion

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Amyotrophic lateral sclerosis (ALS) is characterized by motor neuron degeneration in the primary motor cortex, brainstem and spinal cord. The hexanucleotide repeat expansion in C9ORF72 gene (C9ORF72-HRE) is the most frequent genetic cause of ALS. Since many familial pedigrees showed incomplete penetrance and heterogeneous clinical signs, several genetic factors have been analyzed as possible modifier in ALS. The length of GCG repeat in non-imprinted in Prader-Willi/Angelman syndrome 1 (NIPA1), identified as risk factor for ALS susceptibility, has been investigated as a possible modifier factor for C9ORF72-HRE ALS patientsNIPA1 long alleles frequency was significantly higher in C9ORF72-HRE sporadic ALS carriers (15.2%) (vs 5.5% in all other sporadic ALS cases and 3.9% in controls; Dekker 2016) while no difference was observed in C9ORF72-HRE ALS carriers (3.0%) compared to controls (3.5%) (Van Blitterswijk 2014). Based on this, we investigate the possible role of NIPA1-GCG repeat length as modifier of the C9ORF72 phenotype in a large cohort of 558 Italian ALS sporadic cases and 483 Italian controls. To evaluate the effect of short or long repeat length we dichotomized NIPA1 alleles as 'normal' ((GCG) 7-8), or 'long' (>8 GCG repeats). We didn't observe a higher frequency of NIPA1 long alleles in C9ORF72-HRE carriers (4%) compared to C9ORF72-HRE negative patients (4.4%) and healthy controls (5%). This sample size allowed to replicate the modifier effect observed in the literature (92% power, p.=0.05). In conclusion, we did not confirm a role of NIPA1 repeat length as a modifier factor of the C9ORF72 ALS phenotype.

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P10.43C

SMA-affected individuals with one copy of *SMN2* gene

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Introduction: Spinal muscular atrophy (SMA) is a neurodegenerative disease characterized by loss of lower motor neurons of spinal cord. SMA is caused by mutations in the telomeric copy of the survival motor neuron gene (*SMN1*). Affected individuals with damaged *SMN1* gene seem to have milder form of the disease with increased copy

numbers of the centromeric homological copy of *SMN* gene (*SMN2*) producing only 10% of functional protein. On the contrary in this study an attention has been turned to patients with one copy of the *SMN2* gene.

Materials and methods: Eight SMA patients with pathogenic variants in SMN1 gene and one copy of SMN2 gene have been analyzed. MLPA and sequence analysis have been performed previously. (Tab. 1) **Tab.1** Characteristics of patients in current study

Sample №	Age	SMA Type	*SMN1* copy number	*SMN1*, minor mutation	*SMN2* copy number
690	4 m	1	0	–	1
1485	3 m	1	0	–	1
1510	9 m	1	0	–	1
4291	11 m	1	0	–	1
7600	10 d	0	0	–	1
9664	12 d	0	0	–	1
8589	8 y 8 m	2	1	p.Thr274Ile	1
8826	1 y 7 m	2	1	p.Thr274Ile	1

Results: The SMA types had varied from Type 0 with reduced fetal movements, respiratory failure immediately after birth, lack of the joints mobility and severe muscle weakness to Type 2 "strong". Patients with Types 0 and 1 have had homozygous deletion of the SMN1 gene. And patients with milder SMA type 2 have been compound heterozygotes (with deletion and pathogenic SMN1 variant p.Thr274Ile close to a wild type).

Conclusion: The variability of the SMA phenotypes is observed even with a single copy of the *SMN2* gene. It can be important of the new opportunities in the therapy of SMA.

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P10.44D

Combined therapy using low-dose SMN plus Ncald ASOs further ameliorates disease symptoms in a severe mouse model for spinal muscular atrophy

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Introduction: Spinal muscular atrophy (SMA) is a first genetic condition for which ASO therapy has been FDA- and EMA-approved. SPINRAZA are splice-correcting ASOs targeting *SMN2* RNA which produce increased SMN levels. Despite impressive clinical improvements in some patients, there is still a need for additional therapeutic approaches to treat the full spectrum of SMA patients. Therefore, identifying additional SMN-independent therapeutic approaches are warranted. We identified neurocalcin delta (NCALD) reduction as a protective genetic modifier in asymptomatic *SMN1*-deleted individuals and across species (Riessland et al., AJHG 2017).

Materials and methods: In collaboration with IONIS Pharmaceuticals, *Ncald*-ASOs were developed to reduce NCALD level. 30 *Ncald*-ASOs were generated and tested in cells and adult mice; the three most efficient were tested for tolerability and efficiency in the neonatal Taiwanese SMA mouse model. *Ncald3*-ASO showed the optimal viability, with non-toxic effects and the best decrease of protein expression: 75% in brain and 80% in spinal cord.

Results: Upon optimization, we performed a blinded preclinical study using presymptomatic injection of low-dose SMN+*Ncald*-ASOs compared to SMN+control-ASOs, where *Ncald*- or control ASOs were injected ICV at P2 and SMN-ASOs subcutaneously at P1. Our results showed a significant increase in compound muscle action potential, neuromuscular junction size and proprioceptive input in SMN+*Ncald* treated SMA mice at P21 and P90 and analyses for P180 are in progress.

Conclusion: These findings suggest that a combinatorial approach using SMN-dependent and SMN-independent ASO-therapy - resembling a condition found in asymptomatic individuals - further ameliorates disease symptoms.

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P10.45A

Prenatal diagnosis of spinal muscular atrophy in 2 couples with unusual gene arrangement in the SMA region at 5q13

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Introduction: Spinal muscular atrophy (SMA) is a common autosomal recessive disease with a carrier rate of about 1/50 worldwide. It is characterized by progressive muscle

weakness and atrophy. There are three major types of SMA distinguished by the severity and age of onset. More than 94% of SMA patients, regardless of clinical type, have homozygous absence of *SMN1* gene. We report 2 cases of prenatal diagnosis with previous birth of SMA affected children due to absence of *SMN1* gene.

Materials and methods: Presence of *SMN1* gene exons 7 and 8 in the fetuses was detected by *Hinf*I and *Dde*I digests. Copy numbers of *SMN1*, *SMN2* and *NAIP* genes of both families were further analysed by MLPA (P021 MRC-Holland) to confirm the results and to determine the phase of gene arrangement.

Results:

	Gene	Father	Mother	SMA child	Fetus
Couple 1	*NAIP*	1	3	1	2
	SMN1	1	1	0	1
	SMN2	1	2	2	0
Couple 2	*NAIP*	1	2	1	2
	SMN1	1	1	0	2
	SMN2	1	2	3	0

Conclusions: Both pregnancies were unaffected. One of them was a carrier and both carried no SMN2 genes. MLPA analysis confirmed the phase of gene arrangement for couple 1, but presented more possibilities for couple 2. This finding shows the complexity of gene arrangement due to deletion/gene conversion in the SMA region.

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P10.47CSpinal Muscular Atrophy diagnosis in 150 Iranian families with affected child

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Background: Spinal muscular atrophy (SMA) is highly heterogeneous disorder and the second most common diseases after thalassemia in Iran. Autosomal recessive (AR) forms of diseases are more common in populations with higher rate of consanguineous marriages. Autozygosity mapping is a powerful technique to track the defective gene in consanguineous populations like Iran.

Methods: In this study, all suspected patients to SMA were examined by MLPA to screen for deletion/ duplications of SMN1,2 genes. Subsequently the families with no mutation were investigated by homozygosity mapping with the help of STR markers linked to the DNAJB2, IGHMBP2, SIGMAR1, and PLEHG5 genes. These genes are the responsible for atypical form of SMA with AR inheritance. The patients who showed linkage to the mentioned genes were directly sequence. Finally, Whole Exome sequencing (WES) was done for the 5 patients who did not show any linkage with any of genes in this study.

Result: From 150 studied families, a mutation in SMN1 genes identified in 115 families. Homozygosity mapping in 44 families (29.3%) showed linkage in three families to three different genes. The mutations were in DNAJB2, SIGMAR1, and PLEKHG5 genes. In 5 families tested by WES, 3 families had a pathogenic variant in the TNNT1, TPM3 and TTN genes. ACMG guideline were used to assess the pathogenicity of the new variants.

Conclusion: The use of MLPA in concomitant with STR markers in typical SMA cases can increase the rate of mutation detection with a very low cost.

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P10.48D
Molecular analysis of CAG repeats in five spinocerebellar ataxias: The distribution and reference ranges of SCA1, 2, 3, 6 and 7

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Introduction: The Spinocerebellar ataxias (SCA) is autosomal dominant diseases characterized by heterogeneous group of nuerodegerative disorders with variable expression. CAG repeat expansions in the causative genes have been used for the diagnoses of patients with ataxia. We investigated five types of SCAs in 149 unrelated patients with ataxia to elucidate the distribution of CAG repeats and the mutation spectrum of SCAs in Korea.

Materials and methods: In order to evaluate CAG repeat size ranges of SCAs by capillary electrophoresis analysis, Genomic DNA was extracted from peripheral blood using the Chemagic DNA Blood 200 Kit (Chemagen, Baesweiler,

Germany). Amplified products were injected into an ABI 3500xL Genetic analyzer (Applied Biosystems, Foster City, CA, USA). Amplicon length was calculated by comparison with the GS500-ROX molecular weight standard by using the GeneMarker v.2.2 software (SoftGenetics, State College, PA, USA).

Results: The normal and pathologic CAG repeat size ranges were established at five SCA loci. The total prevalence of the five types of SCAs was 14.8% in the 149 patients with ataxia, regardless of their family history. The most frequent type was SCA 2 (5.6%), followed by SCA 6 (5.4%). SCA1, SCA7, and SCA 3 were less frequent, affecting 3.0%, 2.5%, and 0.9% of the cases, respectively.

Conclusions: We determined the CAG repeat size ranges of each SCA type on normal and pathologic alleles. This study will provide the characteristics of the SCA mutations, and an effective strategy for the molecular diagnosis of SCAs in Koreans.

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P10.49A

Improving coverage of muscle specific genes in targeted massively parallel sequencing

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Introduction: Massively parallel sequencing (MPS) methods have transformed the genetic analysis of neuromuscular disorders. These disorders are both genetically and phenotypically heterogeneous and therefore laborious to study. However, low coverage regions challenge the comprehensiveness of MPS studies.

Our research unit utilises a targeted MPS assay, MYOcap, in clinical diagnostics of myopathy. The aim of this study was to systematically survey the target coverage and especially concentrate on defining the low covered regions in different versions of MYOcap. Furthermore, we wanted to examine the effect of probe design optimisation on boosting the coverage of the target region.

Materials and methods: Approximately 1500 samples had previously been analysed with one of the five versions of MYOcap. Coverage information from the whole cohort was available for this study. Initially, the target region of MYOcap consisted of exons and UTRs of 180 known or putative myopathy-causing genes. MYOcap has since been gradually expanded to target 328 genes. Furthermore, sensitivity of the assay has been enhanced by optimising the probes targeting the low covered regions.

Results: Irrespective of version, the coverage of MYOcap reached at least 20X in 94% of the target region. However, recurrent low coverage (<20X) areas were detected. Optimised probe design improved the coverage for majority of these regions but some low covered areas remained. These are areas that are difficult to sequence or unambiguously map.

Conclusions: There are genomic regions that are resistant to even targeted MPS. Currently, these areas have to be studied by complementary methods.

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P10.50B

A Faroese founder variant in *TBCD* causes early onset, progressive encephalopathy with a homogenous clinical course

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Introduction: Faroe Islands is an archipelago of 18 islands with around 49,000 inhabitants, which is situated between Iceland and Norway in the North Atlantic Sea. The Faroese population originates from a small settlement and several genetic disorders have an increased incidence in the present population due to founder effect, e.g. mitochondrial encephalomyopathy due to a *SUCLA2* variant and Aicardi Goutières syndrome. Variants in a host of structural microtubulin-associated proteins have been identified to cause progressive neurodegenerative disorders. TBCD is one of five tubulin-specific chaperones and is required for

reversible assembly of tubulin-heterodimer. Recently, mutations in *TBCD* have been identified in patients with distinct progressive encephalopathy with a broad clinical spectrum.

Materials and methods: Patients with encephalopathy were collected from families originating from the Faroe Islands. All were tested negative for the Faroese *SUCLA2* founder variant. Whole exome sequencing (WES) was performed to search for a disease-causing variant.

Results: By WES, we found several patients to be homozygous for a *TBCD* missense variant, with a high carrier frequency in the Faroese population. These patients presented with an early-onset, progressive encephalopathy with features of primary neurodegeneration and a homogenous clinical course. We present a detailed description of the variant including functional studies, clinical findings, neuropathology, and MR imaging characteristics of a subset of these patients, adding insight into the phenotype of TBCD-related encephalopathy.

Conclusions: The finding of a Faroese founder variant will allow targeted genetic diagnostics in patients of Faroese descent as well as improved genetic counseling and testing of at-risk couples.

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P10.51C

Possible TDP1 founder mutation underlying spinocerebellar ataxia with axonal neuropathy

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Spinocerebellar ataxia with axonal neuropathy (SCAN1), an autosomal recessive form of hereditary ataxia, has so far been reported in a single extended Saudi Arabian family. We here describe the first independent observation of the condition in two apparently unrelated Omani families. Clinical presentation consisted of early-adulthood onset of progressive ataxia with peripheral axonal motor and sensory neuropathy. Detailed examination revealed significant distal lower limb weakness, fasciculation, amyotrophy and hypo/areflexia and variable degrees of such findings in the distal upper limbs, distal sensory neuropathy in lower limb and high steppage gait with tandem ataxia, in addition to mild finger to nose incoordination and ill sustained gaze evoked nystagmus in some. Identical to the Saudi family, the homozygous missense variation (c.1478A>G or p. His493Arg) located in the second active site of the TDP1 gene (tyrosyl-DNA phosphodiesterase 1), was found to segregate in these two families. SNP array analysis in both probands identified a unique and shared haplotypic background. The single genetic defect against which SCAN1 occurs would explain the relative phenotypic homogeneity observed between affected individuals. In line with the geographic distribution of this rare clinical entity, our observations suggest a common origin of the TDP1 c.1478A>G molecular alteration.

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P10.52D

A new case of rare *TRIP4*-related neuromuscular disease

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Introduction: Autosomal recessive variants in the *TRIP4* gene are known to cause a particular form of congenital muscular dystrophy, type Davignon-Chauveau (OMIM #617066) and a congenital spinal muscular atrophy with bone fractures type 1 (OMIM #616866) as well. Both disorders are very rare. They have been described in only a few families and they have overlapping phenotypic features.

Material and Methods: We report a new case of *TRIP4*-related neuromuscular disease. A 14-year-old female patient, the first child of two healthy apparently non-consaquineous parents, presented with extremely low body weight (BW:23 Kg<<3rd centile), fatigue, muscular weakness and severe scoliosis since the first year of life. Neurological examination revealed muscular weakness, mainly involving truncal and proximal lower limps. Examination of the respiratory, gastrointestinal and cardiovascular systems did not reveal abnormalities. Her intelligence was normal. Whole Exome Sequencing (WES) of the patient and both parents was performed.

Results: A pathogenic homozygous *TRIP4*:c.1678 +1_1678+2insC novel variant was identified in intron 12 of the *TRIP4* gene. The variant is predicted to disrupt the normal splice site as confirmed by splice site prediction

software. It was present in heterozygous state in both parents whose origin is from the same Greek island.

Conclusion: *TRIP4* gene encodes the thyroid receptor-interacting protein 4, one of the four subunits of the tetrameric ASC-1 transcriptional cointegrator complex which is identified to play a significant role in myogenic differentiation and skeletal myotube growth. The use of WES has been proven powerful in expanding the phenotypical and genetic spectrum of rare congenital muscle diseases.

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P10.53A

Evaluation of missense and splicing in silico predictions tools and implementation of an efficient SNV prioritization NGS pipeline for molecular diagnosis of Myopathies and Muscular Dystrophies

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Interpretation of Next Generation Sequencing huge amount of data constitutes the main limitation in molecular genetics diagnosis. In diagnosis of Myopathies and Muscular Dystrophies (MMD), another major issue is to efficiently predict pathogenicity of variants identified in large genes, especially *TTN*, since current *in silico* prediction tools show limitations to predict and rank the numerous variants of such genes.

We evaluated several tools and propose a unique variant prioritization score called MPA. MPA is based on curated interpretation for previously reported variants, biological assumptions, splice and missense predictors to prioritize all types of SNV variants. We validated our approach by comparing MPA versus prediction tools in dbNSFP including CADD using a dataset composed of *DYSF*, *DMD*, *LMNA*, *NEB* and *TTN* variants extracted from expert-reviewed (*missense* $n = 246$; *splice* $n = 190$ *pathogenic variants*) and ExAc database (*missense* $n = 603$; *splice* $n = 3441$ *neutral variants*).

MPA obtained the best annotation rate for missense and splice variants. As MPA aggregates results from several predictors, individual predictors errors are counterweighted, improving sensitivity and specificity of missense and splicing variants prédictions, especially in *TTN*. We propose a sequential use of MPA, beginning with selection of variants with higher scores, followed in the absence of candidate variants, by consideration of variants with lower scores.

We provide scripts and documentation for an free academic use validated annotation and prioritization pipeline. This pipeline is scaled for panel and exome sequencing in molecular diagnosis of MMD.

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P10.54B

UNC13A causes a severe and recognizable phenotype in humans

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We report an infant with encephalopathy, seizures, neuromuscular features, and progressive respiratory failure associated with a homozygous null mutation in UNC13A, further refining the phenotype and confirming its role as a disease causing gene in humans. Our patient was non dysmorphic with normal growth parameters who presented with hypertonia and encephalopathy immediately after birth. EEG revealed burst suppression. MRI of brain non diagnostic. Seizures were uncontrolled and severe global hypotonia developed, with limited movement. A Morgagni diaphragmatic hernia, an umbilical hernia, and

kyphoscoliosis developed in infancy. She had severe constipation and urinary retention. She died at 8 months of respiratory failure and had not met any significant developmental milestones. Autopsy revealed an unusual pattern of myelination of the brain not previously documented. EM of skin revealed lysosomal inclusions. Exome trio analysis revealed a UNC13A variant, c.1188delC p.(Asp397Thrfs*107), homozygous and in trans. UNC13A protein is located in cholinergic neuromuscular synapses and in the majority of glutamanergic synapses in the brain. Mice with Munc13a homozygous null mutations are paralysed and die. Two patients with homozygous mutations have been reported in the literature, with features that may include global delay, microcephaly, seizures, fatal myasthenia with respiratory failure, hypotonia, myopathy. Our patient had progressive hypotonia, myopathy and seizures, and a fatal course with features strikingly similar to the two reported patients. Umbilical hernia, Morgagni hernia, normocephaly and newly reported pathological findings add to the clinical phenotype and confirm homozygous null mutations in UNC13A cause a unique, recognizable, and severe phenotype in humans.

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P10.55C
Molecular characterization in Mexican patients with limb-girdle muscular dystrophy

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Bone ring muscular dystrophies are neuromuscular disorders of genetic origin. Currently, there are more than 30 subtypes of waist dystrophies. Subtype 2 or LGMD2 is a group of entities with an autosomal recessive inheritance pattern. In this study, the genetic profile was characterized through NGS, DNA direct sequencing and MLPA in Mexican patients with LGMD. Genomic DNA was obtained from peripheral blood of 20 patients. Variants were analyzed with SIFT and Mutationtaster softwares; UMD-DYSF was used for dysfelinopathies . In this work, 25% of the patients presented positive findings with the NGS panel and in 15% the diagnosis of the dystrophy subtype was confirmed by molecular study. Of the 20 patients, 2 homozygous patients were identified for dysfunctional dysfunction and an atypical family case with a heterozygous mutation in DYSF. One of the patients was compound heterozygote for calpain and another presented variants of uncertain significance in the *CALPN3* and *DYSF* genes in spite of despite the fact that they fulfilled clinical criteria for muscular dystrophy. NGS is a useful tool for the diagnosis of patients with clinical symptoms of LGMD, even in patients with atypical clinical characteristics. We found novel mutations not previously reported in the literature and a rare mutation in *DYSF* previously described in Ashkenazi Jewish population in two members of a family. The NGS should be implemented as a first-line diagnostic tool in these pathologies

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P11 Multiple Malformation/anomalies syndromes

P11.001A
Detection of concomitant intragenic variants in inherited 16p13.11 microdeletions explaining phenotypic inconsistencies

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The recurrent 1.2-1.5 microdeletion of chromosome region 16p13.11 has long been claimed to be a susceptibility factor for neurocognitive impairment, autism and epilepsy, with highly variable phenotypic manifestations, in a double hit model of pathogenesis. In most cases the deletion is inherited from a healthy parent. Two patients we observed with 16p13.11 microdeletion inherited from a healthy parent presented with a severe phenotype, including severe microcephaly associated with a complex brain malformation, and microcephaly associated with choreoatetosis and

dystonia, respectively. The complex brain abnormalities (corpus callosum agenesis, pachygyria, cerebellar hypoplasia and colpocephaly) in the first case prompted us to perform direct sequencing of the *NDE1* gene. A nonsense variant on the remaining allele was identified, leading to the diagnosis of autosomal recessive type 4 lissencephaly. Our second patient was analyzed by WES (trio analysis). We detected a de novo missense variant in *GABRB2*, which has recently been reported in a group of patients with an overlapping phenotype including choreoatetosis, dystonia, microcephaly and severe ID with white matter abnormalities. Our results confirm that 16p13.11 deletion itself is not sufficient to cause the disease, with two possible opposite scenarios: 1) the microdeletion is involved in the etiology of the observed condition, when it unmasks a recessive variant on the remaining allele (i.e.: hemizygosity of a pathogenic *NDE1* variant); 2) the microdeletion acts as innocent bystander of a different gene variant.

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P11.003C

First case of 19q triplication: clinical and cytogenomic characterization

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Introduction: Intrachromosomal triplications leading to tetrasomy for the corresponding segment is a rare complex chromosomal rearrangement. It has been reported for a few chromosomes but, to our knowledge, there is no case involving chromosome 19 so far. Here we describe a 13 months old girl with 19q terminal triplication, marked hypotonia, developmental delay, dysmorphic features, abnormal skull shape and premature thelarche and pubarche.

Methods and Results: GTW banding technique (550 band level) showed extra material on 19q. As parental karyotypes were normal, SKY analysis was performed. It revealed that the derivative chromosome presented a uniform coloration corresponding to specific sequences of chromosome 19 suggesting a probable duplication 19q13.33q13.43. The FISH pattern on metaphase chromosomes with 19q subtelomeric probes was not conclusive. Microarray analysis identified a 3.7Mb triplication of the 19q13.42q13.43 segment and a 1.7 Mb microduplication at 16p13.11. FISH reanalysis on nuclei confirmed the tetrasomy.

Conclusions: We report the first case of 19qter tetrasomy by intrachromosomal triplication, which was not distinguishable from duplication by conventional cytogenetics, but was clearly identified by microarray analysis. This result allowed us to interpret the signal pattern of FISH on metaphases revealing that the middle repeat of the triplication was inverted. Cytogenetic and cytogenomic characterization of the rearrangement supports the molecular mechanisms postulated for the formation of intrachromosomal triplications. Microarray analysis allowed the detection of a cryptic microduplication at 16p. It is difficult to establish a phenotype-genotype correlation due to the absence of other reported cases and the probable additive effect of the 16p13.11 microduplication.

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P11.004D

Investigation of genetic variants underlying variability in patients with 22q11.2 Deletion Syndrome

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Introduction: The 22q11.2 deletion syndrome (DS) is the most common microdeletion syndrome with an incidence of 1 in 2000 births. Major clinical characteristics are intellectual disability, congenital heart anomalies, immune system defects, velopharyngeal abnormalities, facial dysmorphism and psychiatric disorders. Affected individuals have a hemizygous 1,5-3Mb deletion at chromosome 22q11.2, which includes about 50 known genes. Highly variable expressivity of the clinical features can be observed even in the patients carrying the same deletions. In the project we focused on two of the potential mechanisms underlying this variability: allelic variation within the 22q11.2 region of the non-deleted chromosome and variants in modifier genes outside of the deletion.

Materials and methods: To identify rare and likely phenotype influencing variants Illumina whole exome sequencing (WES) in 50 patients with DS was applied. Detailed phenotyping of all patients was performed to estimate correlation between WES results and specific phenotypes.

Results: Analysis of the hemizygous region of 22q11.2 revealed rare nonsynonymous SNVs in HIRA and MRPL40 genes. In the region outside of the deletion we found 14 variants that may be considered as a probable phenotype modifiers; 7 rare nonsynonymous SNVs in genes associated with congenital heart malformations were found in the group of 34 patients with heart defects (CITED2, MED13L, NKX2-5, NODAL, TLL1, CHD7, MYH6); 6 rare nonsynonymous SNVs and 1 frameshift insertion were consistent with patients' clinical features, including one variant in gene from genetic pathway of 22q11.2 region (CDH15).

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P11.005A

Additional CNVs modifying phenotypes of patients with 22q11.2 Deletion Syndrome detected from exome sequencing data

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Introduction: The most common microdeletion in humans is 22q11.2 deletion syndrome (DS) caused by deletion of a 1.5–3 Mb region at chromosome 22q11.2 and occurring in 1 in 2,000 births. This disorder is associated with variable phenotypes, such as congenital cardiac defects, velopharyngeal abnormalities, characteristic facial appearance, renal anomalies, intellectual disability and psychiatric disorders. One of the potential genetics mechanisms underlying this variability is the presence of additional copy number variant (CNV) elsewhere in the genome.

Materials and methods: To identify CNVs in genome, other than 22q11.2 deletion, CNV calling algorithm CoNIFER was implemented on the exome sequencing data set of 40 patients.

Results: In three patients, additionally to 22q11.2 loss, we found one deletion and three duplication calls that were verified by aCGH. In the first patient showing, unusual for 22q11.2 DS, regression of cognitive functions and dementia, atypical ~3,34 Mb 22q11 deletion encompassing *TUBA8* gene was present. In second, male patient we found 365 kb potentially pathogenic duplication of Xq22.3 encompassing the *PRPS1* gene and exons 1-5 of *MID2* gene that is associated with X-linked mental retardation. In the third patient two additional CNVs with uncertain clinical significance were found: 667 kb deletion of 3p26.3 encompassing exons 1-2 of the *CNTN4* gene and 618 kb duplication of 7q21.3q22.1 including *NPTX2* gene.

Conclusions: In patients with 22q11.2 DS diagnosed by MLPA or FISH further investigation of CNVs is recommended.

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P11.006B

Next-generation sequencing (NGS) of nine candidate genes with custom AmpliSeq in 22q11.2 deletion syndrome patients

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22q11.2 deletion syndrome (22q11.2DS) results from hemizygous 3 Mb deletions of chromosome 22 usually flanked by low copy repeats (LCR) which represents a substrate for aberrant recombination. Even though most 22q11.2DS patients have the same size deletions, the phenotype is highly variable among individuals.

The presence of single nucleotide variants (SNVs) on the remaining allele of 22q11.2 or in other genes outside 22q11.2 region is suggested to have a role in the phenotype variation observed.

We performed Ion Ampliseq in peripheral blood from 30 22q11.2DS patients to sequence the coding regions of nine candidate genes located in and outside the 22q11.2 hemizygous region. We identified 30 SNVs in 3'UTR regions and 21 SVNs in exons, being 12 missense and nine

synonymous SNVs. To predict the pathogenic potential of the SNVs, we performed *in silico* analysis by using the tools Mutation Taster, FATHMM, PolyPhen 2 and SIFT. The SNVs found in the *CRKL*, *PI4KA*, *MAPK1*, *ZFPM2* and *TANGO2* genes led to aminoacid substitution, changes on splicing sites or RNA's polyA tail alteration. Of the 51 variants analyzed, 11 variants were predicted as deleterious, one variant was predicted as protective and 19 variants were predicted to be tolerable to mutations.

Some of our findings were not previously described in the literature, which represents a great potential for further studies and contribution to the field, after validation. The use of gene panels with candidate genes could accelerate the search for genetic modifiers and improve the treatment of 22q11.2DS patients. Financial support: FAPESP, Brazil.

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P11.007C

Novel mutation in MASP1 gene in three new patient with 3MC syndrome

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3MC syndrome encompasses the four autosomal recessive conditions Malpuech syndrome, Michels syndrome, Mingarelli syndrome, and Carnevale syndrome. The main features of these syndromes are facial dysmorphism that includes hypertelorism, blepharophimosis, blepharoptosis, and highly arched eyebrows. Cleft lip and palate, postnatal growth deficiency, cognitive impairment, and hearing loss are also consistent findings. Craniosynostosis, radioulnar synostosis, and genital and vesicorenal anomalies occur in 20 to 30% of cases. 3MC syndrome can be caused by mutations in either COLEC11or MASP1 genes. Recently, COLEC10 gene was discovered as the cause of the disease. The 7,5 months old infant was referred to our genetic policlinic because of her dysmorphic appearance. Frontal bossing, highly arched eyebrows, hypertelorism, blepharophimosis, flattened nasal root, umbilical hernia, sacral dimple were observed in physical examination. Horseshoe kidney was detected on ultrasound. Her sister and brother also had the similar symptoms. Parents were first degree cousins. Pedigree analysis showed autosomal recessive trait. Both the patient and her siblings were diagnosed with 3MC syndrome. Sequence analysis of the MASP1 gene identified a novel not previously described mutation.In this report, we describe three new patients of 3MC syndrome in a Turkish family with a novel mutation of MASP1 gene. Different clinical features of patients, follow-up, management and mutation type of MASP1 gene will be discussed in detail.

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P11.008D

Novel frameshift-mutation in EOGT gene in a female individual with a severe autosomal recessive Adams-Oliver Syndrome with neurological findings and squamous cell carcinoma

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Adams-Oliver Syndrome (AOS) is defined by aplasia cutis congenita (ACC) of the scalp and terminal transverse limb defects (TTLD). The clinical findings are variable, there can be cardiac, neurologic, renal and ophthalmological findings. Several genes have been identified: *ARHGAP31*, *DLL4, NOTCH1*, and *RBPJ* are associated with autosomal dominant inheritance, *DOCK6* and *EOGT* with autosomal recessive inheritance. In autosomal dominant families, the penetrance can be reduced and neurologic findings are very rare. To our knowledge, only 8 families have been described with mutations in *EOGT* to date. Three different mutations were detected so far, 2 missense mutations and a frameshift mutation, which was found in 6 of the 8 families, suggesting a founder mutation in consanguineous families of Arabic ancestry. We here report on a 12 year old girl from Iraq with consanguineous parents. She was born with a severe AOS with a large scalp defect (ACC) which was not treated until recently. Molecular genetic analysis (next generation sequencing) revealed a novel frameshift mutation in the *EOGT* gene, most probably in homozygous constellation. Clinically, the girl showed TTLD on both hands and feet, and she had neurological findings: spastic paresis, epilepsy, microcephaly, and suspicion of intellectual deficits. In the course of treatment, a squamous cell carcinoma was detected, she developed a hydrocephalus. An association between squamous cell carcinoma and AOS has not been described in literature so far. However, this case report shows another hint, that autosomal recessive AOS might be associated with a more severe phenotype.

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P11.009A

Unravelling the genetic architecture in an extensive cohort of Adams-Oliver syndrome patients

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Introduction: Adams-Oliver syndrome (AOS) is a rare developmental disorder, characterized by scalp aplasia cutis congenita (ACC) and transverse terminal limb defects (TTLD). Several causative genes have been discovered over recent years. Autosomal dominant forms of AOS are linked to mutations in *ARHGAP31*, *DLL4*, *NOTCH1* or *RBPJ*, while *DOCK6* and *EOGT* underlie autosomal recessive inheritance. Despite these advances, data on the frequency and distribution of mutations in large cohorts is currently limited. The purpose of this study was to comprehensively examine the genetic architecture of AOS in an extensive European cohort.

Material and Methods: Molecular diagnostic screening of a cohort of 194 AOS/ACC/TTLD probands and their families was conducted using a combination of custom-capture, whole-exome and capillary sequencing analyses.

Results: In total, we identified 63 likely pathogenic mutations, of which 22 were novel, providing a molecular diagnosis 30% of patients. In familial cases, the diagnostic yield was 37%. *NOTCH1* is the major contributor, underlying 10% of AOS/ACC/TTLD cases, with *DLL4* (6%), *DOCK6* (6%), *ARHGAP31* (3%), *EOGT* (3%), and *RBPJ* (2%) representing additional causality in this cohort.

Conclusions: We confirm the relevance of genetic screening across the AOS/ACC/TTLD spectrum, highlighting important but limited genotype-phenotype correlations. The presented cohort offers potential for further in-depth screening and novel gene identification to address missing heritability.

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P11.010B

c.105C>A [p.(Tyr35Ter)] in AIMP2 causes microcephaly, intellectual disability, seizures and spastic quadriparesis

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Introduction: In human and other mammalian cells, a unique large tRNA multi-synthetase complex organizes 9 cytoplasmic aminoacyl-tRNA synthetases consisting of bifunctional, as well as the monospecific tRNA synthetases and 3 non-enzyme factors, namely p43 (*AIMP1*), p38 (*AIMP2*) and p18 (*AIMP3*). The p38/AIMP2 protein is a key component of the multi-ARS complex and is crucial for the assembly of the complex. *AIMP1* has been associated with hypomyelinating leukodystrophy-3 characterized by progressive neurodegeneration, microcephaly, spasticity, coarse facies, progressive contractures, generalized brain atrophy and early death.

Materials and methods: We ascertained two consanguineous families with two affected children each with microcephaly, refractory seizures, intellectual disability and spastic quadriparesis. Brain MR imaging showed atrophy of cerebrum, cerebellum and spinal cord, prominent cisterna magna, symmetric T2 hypointensities in the bilateral basal ganglia and thinning of corpus callosum. Whole exome sequencing was carried out on peripheral leucocyte DNA of the affected individuals from both families.

Results: Whole exome sequencing of three affected individuals from the two unrelated families revealed c.105C>A [p.(Tyr35Ter)] variant in homozygous state in *AIMP2*. The variant is present in a shared homozygous region, likely due to a founder effect.

Conclusion: The phenotype of our patients shares marked similarity with that of mutations in closely related gene, *AIMP1*. We hereby report the first human disease associated with deleterious mutations in *AIMP2*.

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P11.011C

Detection of a somatic mosaic variant in the *AKT3* gene in a patient with clinical diagnosis of Macrocephaly-Capillary Malformation

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Introduction: Activating germ-line and somatic variants in *AKT3* have been reported in 24 patients with hemi/megalencephaly and/or segmental cortical dysplasia. There is also one patient reported with a germ-line variant in *AKT3* and clinically diagnosed with Macrocephaly-Capillary Malformation (MCAP), a syndrome caused by somatic activating *PIK3CA* variants and included in the *PIK3CA* Related Overgrowth Spectrum (PROS).

Materials and methods: We clinically evaluated one patient with a diagnosis of MCAP, and screened by NGS a series of genes associated to the PI3K-AKT-mTOR pathway in blood, buccal swab and affected tissue samples. Candidate variants were validated by pyrosequencing.

Results: The *AKT3* variant c.863C>T; p.(Thr288Ile) was detected in 24% (59/246) of the readings by NGS in skin sample, 21% (49/232) in buccal swab, and 1% (5/775) in blood. The variant is not described in control population, cancer, or patients with PROS. Clinically, the patient had macrocephaly, prominent forehead, widely spaced eyes, capillary malformation in philtrum and right knee, 2-3 cutaneous syndactyly in the feet, hypotonia, lower limb asymmetry, and cerebral anomalies: segmental cortical dysplasia, polymicrogyria, cavum septum pellucidum and cavum vergae, supratentorial ventriculomegaly, and hydrocephalus. The combination of all these features is compatible with MCAP.

Conclusions: We describe the first patient with MCAP and a somatic mosaic variant in *AKT3*. Functional analysis is underway.

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P11.013A

Axenfeld - Rieger syndrome - etiology for Mitral and Aortic valve insufficiency

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Objective: To present a child with features of Axenfeld-Rieger syndrome, initially suspected of rheumatic fever carditis. Although rare, Axenfeld-Rieger syndrome is an autsomal dominat disease, known to be associated with valvular and other cardiac disease. When the PITX gene is involved, malformations of several different organs is possible.

Methods: A 14 year old boy was admitted for cardiac murmur and fatigue after minor exertion.

Results: The patient had a good general state, with a Grade II Aortic and Mitral heart murmur. The ECG revealed sinus rhythm, slight tachycardia. Echocardiography showed: a Grade II Aortic and Mitral regurgitation. Lab tests were normal, without any signs of streptococcal infection: ASLO, throat swab and blood cultures were normal. Antibodies for collagen disease were negative. Therefore we excluded rheumatic fever, endocarditis and collagen disease. The particular facial aspect with discrete exophthalmia of the left eye, coloboma with a particularly shaped pupil and iris anisocorya made us send the patient for an ophthalmic exam, where Axenfeld-Rieger syndrome was suspected. Recently, mutation of FKHL7 gene was proved to cause defects of the heart valves. Genetic tests are in progress. The patient was discharged after bacterial endocarditis prophylaxis was implemented.

Conclusions: Usually, Mitral and Aortic valve insufficiency is presented in rheumatic fever, bacterial endocarditis or collagen disease. In this case, after we excluded them, due to the eye involvement with corectopia pupil of the iris Axenfeld-Rieger syndrome was the correct diagnose. The lack of cases reported in literature, determined us to present this patient.

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P11.014B

Identification of a new mutation confirms the implication of *IFT27* in Bardet-Biedl Syndrome (*BBS19*)

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Background: Bardet-Biedl Syndrome (BBS; MIM 209900) is a recessive and genetically heterogeneous ciliopathy characterized by postaxial polydactyly, retinitis pigmentosa, obesity, hypogonadism, cognitive impairment and kidney dysfunction. At the time of this study, 21 BBS genes were identified, with the last reported ones being found in one or very few families.

Methods and Results: Exome sequencing was performed in a child presenting with typical BBS features (retinitis pigmentosa, postaxial polydactyly of four extremities, brachydactyly, obesity, hypogonadism with micropenis, cognitive impairment, neurosensorial deafness and atrioventricular septal defect) as no mutations were identified in known BBS genes by different molecular approaches (Sanger Sequencing of *BBS1*, *BBS10* and *BBS12;* Targeted High-Throughput Sequencing including *BBS1* to *BBS16* genes). Two mutations were found in *IFT27* gene (NM_ 006860: c.[107A>G];[352+1G>T], p.[Tyr36Cys];[?]). Familial segregation was consistent with autosomal recessive inheritance as each parent carried one mutation. Functional studies on fibroblast cells of the patient showed that the c.352+1G>T mutation led to the exon 5 skipping. *IFT27* mutations have been already reported once in a consanguineous BBS family (Aldahmesh *et al*., 2014) with a typical presentation (obesity, mild intellectual disability, polydactyly of all extremities, renal failure, retinitis pigmentosa and hypogenitalism).

Conclusions: This is the second report of *IFT27* mutations in BBS patients confirming *IFT27* as a BBS gene (*BBS19*). This report confirmed also the implication of IFT-pathway in BBS pathogenesis as previously reported with mutations in *IFT27* and secondarily in *IFT172* (Bujakowska *et al*., 2015).

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P11.015C

International consensus group statement on Beckwith-Wiedemann syndrome

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Beckwith-Wiedemann syndrome (BWS), a human genomic imprinting disorder, is characterised by phenotypic variability that might include overgrowth, macroglossia, abdominal wall defects, neonatal hypoglycaemia, lateralized overgrowth and predisposition to embryonal tumours. Delineation of the molecular defects within the imprinted 11p15.5 region can predict familial recurrence risks and the risk (and type) of embryonal tumour. Despite recent advances inknowledge, there is marked heterogeneity in clinical diagnostic criteria and care. An international consensus group agreed upon 72 recommendations for the clinical and molecular diagnosis and management of BWS, including comprehensive protocols for the molecular investigation, care and treatment of patients from the prenatal period to adulthood. The consensus recommendations apply to patients with Beckwith-Wiedemann spectrum (BWSp), covering classical BWS without a molecular diagnosis and BWS-relatedphenotypes with an 11p15.5 molecular anomaly. The consensus group recommended a tumour surveillance programme targeted by molecular subgroups (though it recognised that surveillance might differ according to the local health-care system e.g. in United States) and the results of targeted and universal surveillance should be evaluated prospectively. International collaboration,including a prospective audit of the

results of implementing the consensus recommendations, is required to expand the evidence base for the design of optimum care pathways

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P11.016D

Myopia, developmental delay and a new mutation in ASXL1: a case report of Bohring-Opitz syndrome

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Introduction: Bohring-Opitz syndrome (BOS) is a rare syndrome characterized by intrauterine growth retardation, poor feeding, profound mental retardation, trigonocephaly, dysmorphisms and a typical posture with elbow flexion and wrist deviation. Only 30 cases have been reported and 20 mutations were found. Based on these cases, diagnostic criteria have been set up for clinical diagnosis.

Case report. Our patient is a 2-year-old boy, born at term to healthy, non-consanguineous Caucasian parents. During the pregnancy, a vesicoureteral reflux was found. The post-natal history was charachterized by: profound neurodevelopment delay and failure to thrive (height on the 10th centile, weight and HC below the 3rd centile); severe myopia (diagnosed at three months); microcephaly and trigonocephaly; hypertelorism, upslanting palpebral fissures, exophthalmus, flat nasal bridge, forehead nevus flammeus and BOS posture. These findings led to a clinical diagnosis of BOS. In agreement with the clinical data, the analysis of the *ASXL1* gene (NM_015338.5) revealed a *de novo* frameshift mutation (c.4127dupG, p.Pro1377Serfs*3). This mutation was not previously reported on the online databases for healthy (dbSnp, 1000g, ESP6500, Exac, gnomAD) and affected (HGMD professional) individuals nor described in scientific literature, but considering its position and type it is considered likely pathogenetic.

Conclusion: We report a further case of BOS with a novel mutation in *ASXL1* gene. Our case enlarges the knowledge about the clinical and molecular data of BOS and confirms the previously reported genotype-phenotype correlation. We are performing functional studies about this mutation in order to discover if the RNA transcript is degraded.

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P11.017A

Efficient CrispR/Cas9-based nucleotide editing to model cardiovascular anomalies of Cantú syndrome in zebrafish

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Cantú Syndrome (CS) is a rare genetic disorder caused by gain-of-function (GOF) mutations in genes encoding the pore-forming (Kir6.1, *KCNJ8*) and regulatory (SUR2, *ABCC9*) subunits of an ATP-sensitive potassium (KATP) channel. CS is characterized by facial anomalies, hypertrichosis, extensive cardiac abnormalities and dilated cerebral blood vessels. CS is debilitating with no specific therapy available. Hence, we applied a novel CrispR/Cas9-based genome editing approach to create CS zebrafish models for therapeutic drug screening.

We efficiently introduced three CS-specific point mutations in *abbc9* and *kcnj8* of zebrafish by combining the CrispR/Cas9 system with a short template oligonucleotide harboring the site of mutation. To demonstrate functional validity, we performed live high-speed video-imaging of zebrafish heart and cardinal vein to assess cardiovascular function at 5 days post fertilization. Additionally, cerebral blood vessels were examined for dilations in live *kcnj8* mutants in a Tg(*kdrl:GFP*) transgenic background.

Analogous to CS patients, knock-in fish reveal significantly enlarged ventricles with enhanced cardiac output, contractility and development of pericardial edema. A significantly reduced vein blood flow velocity can be associated with diminished vascular tone reported in patients. Additionally, *kcnj8* mutant fish display distinct

cerebral vasodilation in a structure resembling the human circle of Willis.

We developed a novel technique to establish CS-specific zebrafish that closely model cardiovascular features and therefore open the possibility of phenotyping-based drug screening potentially repurposing sulfonylureas already clinically applied to inhibit GOF KATP channels involved in neonatal diabetes. Consequently, future studies in our model will improve understanding and clinical management of CS.

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P11.018B

Structural and functional differences in *PHOX2B* frameshift mutations underlie isolated or syndromic congenital central hypoventilation syndrome

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Introduction: heterozygous mutations in the *PHOX2B* gene cause congenital central hypoventilation syndrome (CCHS), characterised by defective autonomic control of breathing. CCHS can be isolated or syndromic (i.e., associated with other autonomic dysfunctions such as Hirschsprung disease (HSCR) and neuroblastoma (NB)). Among *PHOX2B* mutations, polyalanine expansions are mostly associated with isolated CCHS, whereas frameshift mutations (FS) with syndromic CCHS. Our study aimed at identifying the molecular mechanisms underlying genotype-phenotype correlations and the predisposition to the severe associated diseases.

Materials and methods: *PHOX2B* FS mutations identified so far were classified in terms of frame change, protein translation, inheritance and clinical associations. Furthermore, we analysed the functional effects of FS mutations in terms of promoter transactivation of the genes involved in autonomic nervous system (ANS) development and the pathogenesis of HSCR and NB.

Results: the type and the position of translational frame affect the protein structure, the predisposition to HSCR and/or NB, and pattern of inheritance: the majority of inherited mutations occur upstream the polyalanine region, and mutations in the terminal part of the protein are more severe and penetrant. Accordingly, the transcriptional dysfunction occurs with the mutations associated with syndromic and severe CCHS.

Conclusions: our classification is useful for the genetic counselling and our data show that the functional differences among the mutations belonging to different frame subgroups are the underlying cause of the ANS disorders associated with CCHS.

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P11.020D

Neuroradiologic abnormalities in CHARGE syndrome and guidelines for cranial imaging

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Introduction: CHARGE syndrome is a rare congenital malformation syndrome (6 per 100,000 newborns) with high and variable comorbidity. As a result, clinicians may struggle to provide comprehensive care. As patients with CHARGE are at risk for peri-anesthetic complications, it is paramount to combine necessary imaging procedures. To enable efficient and high quality imaging, we used recommendations from literature and data from our CHARGE cohort to propose a guideline for cranial imaging in CHARGE syndrome. Methods: We evaluated MRIs of 38 CHARGE patients. Additionally, we performed a structured literature review to examine all existing advice regarding cranial imaging.

Results:

Neuroradiologic abnormalities in CHARGE cohort	
Vestibular system a/dysplasia	100%
Clivus abnormalities	84%
Olfactory system a/hypoplasia	76%
Cerebellar abnormalities (vermis hypoplasia, foliation defects, other)	53%
Wide lateral ventricles	30%
Frontal hypoplasia	27%
abnormalities of corpus callosum (n = 2), of myelinisation (n = 2), of gyration pattern (n = 4), hippocampal malrotation (n = 3)	incidental (<15%)

Recommended imaging from literature comprises a temporal bone CT and MRI of the brain, labyrinth, olfactory structures, pituitary gland and basiocciput.

Conclusions: Cranial abnormalities are very common in CHARGE syndrome and may aid in diagnosis or require specialized treatment. We propose an imaging protocol in which we recommend combining a temporal bone CT and a comprehensive MRI in 3 directions and describe dedicated sequences to assess abnormalities seen in CHARGE syndrome.

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P11.021A

Medical management of chromosome 18 abnormalities

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Management of genetic conditions is complicated by variable expression and penetrance. These factors are compounded in the case of chromosome abnormalities, making binary designations such as "pathogenic" and "benign" wholly insufficient to inform management of these conditions. When assessing the potential implications of copy number variants involving multiple genes, clinicians must quickly assess which genes are most relevant and what the likelihood is of each of the associated findings in order to devise appropriate management and screening recommendations. With this end in mind, we have developed a set of annotated maps designed as four separate and customized tracks on the UCSC Genome Browser:

(1)

gene hemizygosity (potential to produce phenotype when hemizygous)

(2)

gene suprazygosity (potential to produce phenotype when duplicated)

(3)

phenotype hemizygosity (regions associated with specific haploinsufficient phenotypes)

(4)

phenotype suprazygosity (regions associated with specific excess phenotypes)

Gene Classification	# Hemizygous	# Suprazygous
No clinical effect	156	147
Risk factor	17	5
Conditional	45	1
Low penetrance	8	1
Causal	15	1
Lethal	0	0
Unknown	23	108
Phenotype Classification	**# Hemizygous**	**# Suprazygous**
Non-dosage mechanism	3	3
Low Penetrance	37	3
Causal	12	2
Lethal	2	0
Unknown	25	25

These classifications are refined and updated as new data become available. This tool will help clinicians quickly sift through the data available on each of the genes on chromosome 18, determine their relevance, and formulate an appropriate screening and management plan for individuals with chromosome 18 conditions. Supported by the Chromosome 18 Registry & Research Society.

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P11.022B

Maternal uniparental disomy 22 has possible impact on the phenotype: A case report and literature review

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Introduction: Only several cases of uniparental disomy of chromosome 22 (UPD 22) were published, thus far.

Majority of reported patients did not have typical clinical features, with the exception of two cases, who both had a ventricular septal defect (VSD).

Material and Methods: We present a newborn girl, who was born after first pregnancy of a 40 years old healthy mother. The pregnancy had been monitored due to high levels of serum free-hCG detected during antenatal screening and intrauterine growth retardation of the fetus. The child was born at 34 weeks of gestation with birth weight of 1490 g. Cardiologic examination revealed VSD and the child exhibits facial dysmorphism comprising broad nasal bridge, low set dysplastic ears and preauricular pits. Nonetheless, postnatal neurologic-, ophthalmologic- and otoacoustic emission examinations did not reveal any other abnormalities. A full trisomy 22 in placental tissue was detected, while examination of peripheral blood revealed karyotype 46,XX with no evidence of trisomy 22. Maternal UPD 22 was confirmed in follow up cytogenetic examination.

Conclusions: Based on literature review, and considering the presence of VSD, dysmorphic features in our case, low frequency (and eventually tissue specific) trisomy 22 is still possible. Clinical findings of our patient with full placental trisomy 22 and maternal UPD 22 in lymphocytes will be discussed and compared to previously published cases.

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P11.023C

Whole-exome sequencing in patients suspected to have ciliary disorders has a high diagnostic yield and reveals novel candidate genes

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Introduction: Ciliopathies are clinically and genetically highly heterogeneous conditions, which challenges establishing the accurate molecular diagnosis. This study aims to improve the diagnostic yield by employing whole-exome sequencing (WES) to study the potential genetic causes in patients that clinically resemble a ciliopathy phenotype.

Materials and methods: WES was performed in 166 unrelated patients with a clinical phenotype suspected to be a ciliopathy. In a first step, single-nucleotide and copy-number variants (CNVs) were analyzed within 140 known ciliopathy-related genes. Subsequently, exome-wide analysis was offered in unsolved cases.

Results: Analysis of the ciliopathy-gene panel revealed likely causative variants in 42 highly suspicious ciliopathy patients (diagnostic yield 25%), which included CNVs in *BBS1* and *EVC*. Exome-wide analysis revealed a diagnosis in 19 additional patients (11%), in whom there was often a moderately high suspicion for a ciliopathy. This resulted in a total diagnostic yield of 37%. Furthermore, various potentially relevant mutations were identified in an additional 51 patients (potential total yield up to 67%). This included various interesting candidate genes including *KCTD3*, *DNHD1*, and *KIF14*.

Conclusions: In conclusion, WES analysis has a diagnostic yield of 37% in patients suspected to have a ciliopathy. Moreover, various interesting candidate genes were identified, that could potentially increase the yield to up to 67%. This indicates that WES starting with ciliopathy gene panel analysis followed by exome-wide analysis is a powerful diagnostic tool for identifying the genetic cause in these patients.

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P11.024D

Analysis of*RUNX2*mutations in four Turkish patients with Cleidocranial Dysplasia

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Cleidocranial dysplasia (CCD) is a rare skeletal dysplasia that presents with classic triad of delayed closure of the

cranial sutures, hypoplastic or aplastic clavicles and dental abnormalities. The frequency of CCD is one in 1.000.000 and is inherited in an autosomal dominant manner with mutations in *RUNX2* gene. It is reported that 65% of the mutations occur *de novo*, though gonadal mosaicism in either parent could not be ruled out. Diagnosis of CDC is based on the typical clinical and radiographic findings supported by determined heterozygous pathogenic variant in *RUNX2*. In this presentation, we report four Turkish children presented with classical findings of CCD and their molecular genetic test results.Two patients had missense (c.569G>A, p.R190; c.577C>G, p.R193G), one patient had novel frame shift(c.443_445delTACCAGATGGGAinsG; p.V148Gfs*9) and one patient had gross deletion of exons 6-9. The aim of the present report is to increase the awareness of CCD and discuss genotype and phenotype correlation.

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P11.025A

Identification of clinical and functional disorders associated to *VPS13B* mutations in Cohen Syndrome

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Cohen syndrome (CS) is a rare autosomal recessive disorder caused by mutations in the *VPS13B* gene, which encodes a protein of the Golgi apparatus membrane. Intellectual deficiency, retinopathy, abnormal fat distribution around the waist, and neutropenia are CS main clinical features. For the last 10 years, thanks to clinical and functional studies, we focused on the improvement of CS diagnosis and patients' follow-up. Clinical studies allowed us to show that between 2 and 6 years old, CS children already present with typical facial features, which can help to shift the diagnosis towards CS. We also demonstrated that patients have a high risk of developing type II diabetes and cardiovascular diseases, as 70% of them have low HDL level. Functional studies demonstrated that VPS13B invalidation leads to accelerated adipocyte differentiation, consequent to an increased response of cells to insulin stimulation. Later fat-full VPS13B deficient cells (CS fibroblasts or *VPS13B*-invalidaded by siRNA technology) became resistant to insulin, matching the clinical studies and the risk of type 2 diabetes. We also showed that VPS13B deficient cells have a disorganized Golgi apparatus, which is associated to a strong defect in protein glycosylation. Furthermore, absence of early endosome and presence of enlarged lysosomes suggest a crucial role of VPS13B in endosomal-lysosomal trafficking. All these results allowed us to edit new clinical recommendations for the CS patients' follow-up and will help us to understand other CS symptoms such as retinopathy and neutropenia that we are now exploring.

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P11.026B

Complex chromosomal rearrangement associated to premature ovarian failure

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Correct molecular characterization at nucleotide resolution in complex rearrangements is an important strategy to find genetic mechanisms associated with the patients' phenotypes. We describe a female patient, with secondary amenorrhea, a reciprocal translocation between chromosomes X and 2, and two ~200 kb gains from material of both chromosomes involved in rearrangement. Breakpoints were mapped by 10x Genomics Chromium technology, a NGS-based method using linked reads, combined to library preparation and target enrichment using a premium bait design (Agilent SureSelect) containing "bridging baits", enriched for exonic regions. Translocation breakpoints were validated at nucleotide level by low-coverage whole genome sequencing. Our results suggest a complex rearrangement structure, including a tandem duplication in the translocation junction at the derivative X-chromosome. The *SEPT6* gene was found disrupted at one of the X-chromosome breakpoints. *SEPT6* is coexpressed with genes related to oocyte maturation during metaphase II, and the X-chromosome gain encompasses two genes (*RPL39* and *UPF3B*) highly expressed in the ovarian tissue, that could be also related to the phenotype. However, *SEPT6* knockout mice's phenotype does not include subfertility, so its pathogenicity could not be confirmed. Nevertheless, other mechanisms, as position effect and TAD disruptions, could be related to the premature ovarian failure found in patient.

Thus, the junction point sequencing at nucleotide level provided detailed information about pathogenic mechanisms possibly related to ovarian development and function. The improvement of linked-reads sequencing technologies can facilitate rearrangements elucidation, representing remarkable advancement for cytogenomics research and clinical diagnostics.

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P11.027C

Disruption of *WDR26* by a translocation breakpoint confirms its causal role in Skraban-Deardorff and 1q41q42 microdeletion syndromes

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Microdeletions or contiguous gene syndromes (CGSs) are characterized by variable complex clinical phenotypes caused by hemizygosity of contiguous genes, defined mainly by a common deletion region, or of a major causal gene locus. Identification of breakpoints at nucleotide resolution of balanced chromosomal rearrangements localized within these CGS regions constitutes a key strategy for definition of the phenotypically important genes. The aim of this study is the identification of molecular alterations responsible for an extremely complex clinical phenotype resembling 1q41q42 microdeletion syndrome (coarse facial features, severe developmental delay, congenital heart disease and congenital microcephaly) presented by an individual with a t(1;3)(q42.11;p25.3)dn. Translocation breakpoints were localized and confirmed by large-insert whole-genome and Sanger sequencing, respectively. The 1q42.11 breakpoint disrupts exon 12 of *WDR26*, recently reported as the causative gene of the autosomal dominant Skraban-Deardorff syndrome (SKDEAS, OMIM #617616), with clinical features that almost completely overlap the 1q41q42 microdeletion syndrome. WDR26 is WDR domain-containing protein presumably involved in multiple disease-associated signalling pathways. The 3p25.3 breakpoint disrupts IVS 1 of the *ATP2B2* (OMIM *108733), reported as a modifier of the autosomal recessive deafness-12 (DFNB12, OMIN #601386). The proband's clinical features basically confirm the phenotypic overlaps between SKDEAS and the 1q41q42 microdeletion syndrome. In conclusion, disruption of *WDR26* by the 1q42.11 breakpoint most likely leads to its haploinsufficiency due to nonsense mediated RNA decay, resulting in a complex clinical phenotype, basically matching both SKDEAS and the 1q41q42 microdeletion syndrome. Therefore, we confirm its major causative role in these phenocopy syndromes.

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P11.028D

Genotype-phenotype correlations in six patients carrying two large CNVs: A two-hit model as one of the possible explanations for phenotypic variability in genomic disorders

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Genomic rearrangements represent an important source of genetic variability. Rare, recurrent copy-number variants (CNVs) of pathogenic significance, termed genomic disorders, were identified in persons with a characteristic set of clinically recognizable features.

However, some CNVs are associated with genomic disorders with extreme phenotypic heterogeneity. For example, 16p11.2 deletion has been associated with intellectual disability, obesity, schizophrenia, and 1% of sporadic cases of autism. Girirajan and Eichler (2010) proposed a 'two-hit' model in which a first hit (e.g. 16p12.1 deletions) in concert with a secondary hit (e.g. genetic, epigenetic or environmental insult) results in a more severe phenotype.

We present genotype-phenotype correlations in six patients carrying two large CNVs known to be associated with a genomic disorder or potentially associated with disease. Array CGH revealed one large deletion (1,7 Mb

15q13.2q13.3 deletion, 2,9 Mb 13q21.1q21.2 deletion, 7,8 Mb 5q14.3q15 deletion, 4,8 Mb 15q11.2q13.1 deletion, 1,3 Mb 17p12 deletion, 2,6 Mb 22q11.2 deletion) in all six patients. As additional CNVs we identified: 506 kb intragenic duplication of *CNTN4* gene, 2 Mb 13q21.33q22.1 deletion, 1,6 Mb 16p13.11 deletion, 445 kb deletion of *CHRNA7* gene, 452 kb 15q11.2 duplication and 1,7 Mb 16p13.11 duplication). All findings were confirmed by FISH and we also investigated the parental origin of chromosomal rearrangements.

In our six cases, the second hits were inherited maternally or were *de novo*, and more often noted in phenotypically variable diagnoses than syndromic disorders. In summary, additional CNVs can act as genetic modifiers, which may contribute to the variable phehotypes of genomic disorders.

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P11.029A

Phenotype Assessment of a Brazilian Cornelia de Lange Syndrome (CDLS) cohort

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Introduction: Cornelia de Lange syndrome (CDLS) is a rare genetic disorder caused by mutations in five different genes (*NIPBL, SMC1A, HDAC8, SMC3, RAD21*). The disorder has a wide clinical variabilty, thus our objective is to assess the characteristics of 110 Brazilian patients.

Materials and methods: A questionnaire was filled by parents from the Brazilian CDLS association.

Results: Maternal age at conception varied from 15 to 42y (mean 27.4 y) and 50% were the first-born child. 42/110 (38.2%) had clinical intercurrence during pregnancy. 73/110 (66.4%) were born by c-section, and 47/110 (42.8%) were premature. Birth weight varied from 643 to 3740g (mean 2126g) and birth lenght from 28 to 51cm (mean 42.6cm). The age of clinical diagnosis varied from birth to 38y (mean 1.98 y, median 0.5 y). The current age varies from 1 mo to 43 y (mean 11.2y, median 8y). The main clinical findings are synophris (88.2%), feeding dificulties (81.1%), hypertrichosis (76.4%), short stature (72.7%), gastrointestinal malformations (65.5%), heart anomalies (36.4%) and limb defects (33.6%). All patients presented neurologic developmental delay, and 73.6% also had motor developmental delay. 76/110 (69.1%) have listening comprehension and 44/110 (40%) have speaking ability. 22/110 (20%) are capable of using words, 22/110 (20%) short sentences, and 9/110 (8.2%) are capable of reading and writing.

Conclusion: The questionnaire was a pioneer in CDLS patients and this study provides better understanding of the clinical aspects of the disease, improving health assistance. Half patients were first-born child, reinforcing the importance of genetic counselling.

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P11.030B

***Drosophila melanogaster* as a model to study WNT pathway alteration in Cornelia de Lange Syndrome**

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Introduction: Cornelia de Lange syndrome (CdLS) is a rare genetic disorder affecting neurodevelopment, gastrointestinal and musculoskeletal systems. CdLS is caused by mutations within *NIPBL*, *SMC1A*, *SMC3*, *RAD21*, or *HDAC8* genes. These genes codify for the cohesin complex, a multiprotein structure playing a role in chromatid adhesion, DNA repair and gene expression regulation. It has been demonstrated that a strong correlation exists between cohesin complex function and WNT signalling. Recently, it has been observed that chemical activation of the WNT pathway in *nipblb*-loss-of-function zebrafish embryos and in *NIPBL*-mutated patient fibroblasts rescued the adverse phenotype. Both embryos and fibroblasts present similar patterns of canonical WNT pathway alterations and CCND1 downregulation.

Materials and methods: *Drosophila melanogaster* is an inexpensive model to study CdLS and to screen *in vivo* for therapeutic compounds. Therefore, we have selected fly strains mutated in *nipped-B* and *hdac3* genes (respectively *NIPBL* and *HDAC8* in humans) for assessing the existing correlation between cohesin complex and WNT pathway and to screen for chemicals that revert the CdLS associated-phenotypes efficiently.

Results: We have confirmed that mutated flies weight 5% less than wild type. Moreover, we have tested lithium chloride (LiCl) as WNT activator, demonstrating that 250mM is the highest concentration tolerated.

Conclusions: Hence, we hypothesize that WNT pathway activation could improve mutant phenotype. We will be testing different doses of LiCl and other WNT activator to assess whether some of those chemical compounds could revert the syndrome-associated phenotype.

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P11.031C
Patient with a novel variant in *CREBBP* exon 31 and without a typical Rubinstein-Taybi phenotype

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We report a 17 years old patient with an in-frame deletion in *CREBBP* but without Rubinstein-Taybi phenotype.

The patient presented with developmental delay, severe intellectual disability, ventricular septal defect, talipes, inguinal hernia, peno-scrotal fusion, brain anomalies, feeding difficulties, self-injurious behavior. Our first diagnostic hypothesis was Cornelia de Lange syndrome, but extended sequencing panels of known associated genes (including *NIPBL*, *SMC1A*, *SMC3*, *HDAC8*, *RAD21*) detected no pathogenic variants. By subsequent whole genome sequencing we identified a small deletion in exon 31 of *CREBBP*. Variants in *CREBBP* were originally described as the genetic cause of Rubinstein-Taybi syndrome (RSTS). This in-frame variant, NM_004380: c.5518_5544del (p.Val1840_His1848del), was found in 32% of the reads and confirmed by Sanger sequencing in both peripheral blood and fibroblasts. Segregation analysis on the parents revealed that the variant occurred de novo in the patient and can be classified as pathogenic according to ACMG standards.

Recently, individuals with missense variants in exons 30-31 of *CREBBP* without typical RSTS phenotype were described. All of them show atypical facies and variable features including short palpebral fissures, telecanthus, short nose with depressed bridge, anteverted nares, short columella and long philter. They also show psychomotor development delay (11/11), behavioral anomalies including self-injury (8/11), microcephaly (7/11), feeding difficulties (7/11), transmissible hypoacusia (7/11), short stature (5/11), recurrent infections (5/11), large 1st toe (3/11).

In conclusion, we report a novel variant in *CREBBP* resulting in an atypical phenotype, different from RSTS and with only some resemblance to the 11 atypical cases described with missense mutations in exons 30-31.

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P11.032D
Genetic and neuroradiological clinical correlations in Cri Du Chat syndrome

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Introduction: Individuals with 5p deletions were first reported in 1963 by Lejeune as having Cri-du-chat syndrome. The incidence is estimated to be 1:15,000 to 1:50,000 live births and 1:350 among individuals referred for Intellectual Disability (ID). 5p deletions can be telomeric or interstitial and occur at different breakpoints, ranging in size from the telomeric region at band 5p15.3 (5 Mb) to nearly the whole short arm of chromosome 5 (40 Mb). Cri-du-chat syndrome is observed in patients with deletions encompassing a critical region between 5p15.2 and 5p15.3, first defined by Niebuhr in 1978. The classic phenotype includes a characteristic cry, peculiar facies, microcephaly, growth retardation, hypotonia, speech and psychomotor delay and ID. There is a wide spectrum of clinical manifestations that can be attributed to differences in size and localization of the 5p deletion.

Results: Ten patients with 5p deletions have been included in the present study (6 males and 4 females, age ranging from birth to 28 years). Brain and brainstem MRI was performed on all patients. The deletions were characterized by array-CGH. MRI findings included isolated pontine hypoplasia, vermian hypoplasia, ventricular anomalies, abnormal basal angle, widening of cavum sellae,

increased signal from white matter, corpus callosum anomalies, abnormal cortical development.

Conclusions: Several critical regions related to some of the main features (such as the cry, the peculiar facies, the developmental delay) have been identified. The aim of this study is to further define the genotype-phenotype correlations in 5p deletion syndrome with particular regards to the specific neuroradiological findings.

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P11.033A

Novel Mutations in *CTNND1* Cause Cranial Neural Crest Associated Anomalies and Neurodevelopment Disorders

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The aim of this project was to identify novel pathogenic gene variants in children with multiple unexplained phenotypes, as part of an on-going Cleft-Tooth Anomalies Study taking place at the South Thames Cleft Unit at St Thomas' Hospital, using whole exome sequencing. We have identified four unrelated patients with novel de novo variants in the *CTNND1* gene.

Although CTNND1 is a well-known protein that plays crucial developmental functions, human variants have not been discovered until recently, with a report of three variants in patients with Blepharocheilodontic syndrome (BCD). Otherwise, little is known about the phenotypes that are associated with mutations in this gene.

We have initially discovered a *CTNND1* gene variant in a patient with unexplained craniofacial anomalies including cleft lip/palate and oligodontia, cardiac defects, autism (ASD) and other phenotypes suggestive of a yet unexplored syndrome. In addition, three more patients with variants in *CTNND1* were found in the Deciphering Developmental Disorders Study (DDD) UK, one of which shares the same variant as our patient. He presents with very similar craniofacial dysmorphism, ventricular septal defect and neurodevelopmental problems.

As a group, these children with *CTNND1* variants present with neurodevelopmental disorders and craniofacial dysmorphisms, including orofacial clefts, and heart defects; their phenotype is different from BCD. Moreover, the results of our functional assays suggest that *CTNND1* is a neurocristopathy gene due to its effect on structures of neural crest origin, and potentially a key oral clefting gene.

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P11.034B

Mutations in epigenetic and transcriptional regulation genes cause majority of human developmental disorders

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Multiple congenital anomaly with dysmorphic features, heart defects and/or neurodevelopmental manifestations is a group of phenotypically and genetically heterogeneous developmental disorders (DD) affecting ~2-5% of children. We recruited 228 unrelated patients via broad-reaching collaboration, systematically phenotyped these individuals, and undertook a focused study using exome sequencing. We successfully identified causal mutations in known Mendelian genes for 35.1% of kindreds (n = 80). In addition, we discovered likely pathogenic variants in 5 genes not previously implicated in DD in 3.1% of patients (n = 7). The identified mutations were mostly *de novo* dominant (n = 56, 76%), consistent with the majority of cases arise sporadically without familial occurrence, but also X-linked or autosomal recessive (n = 21, 24%). 58.6% of these 87 resolved kindreds are due to mutations in genes involved in transcriptional processes and related epigenetic modification pathway, including *TP63* (2), *SMARCE1*, *ACTB*, *DDX3X*, *FOXP1*, *FOXC1*, *SMC1A*, *SF3B4* (2), *EFTUD2*, *EP300*,

MED13L (2), *KANSL1*, *SOX2*, *ASXL3*, *CTNNB1* (2), *CTNND1*, *STAG2*, *TBR1*, *MAF*, *CUL4B*, *SATB2* (2), *ADNP* (2), *HNRNPK*, *HIVEP2*, *TFAP2A* (2), *MLL*, *MLL2*, *DEAF1*, *GLI3*, *KMT5B*, *KAT6A*, *KAT6B*, *H3F3A*, *MED12* (7), *GTF3C5*, *NKAP*, *CHD3*, and *U2AF2*, which highlights the fundamental roles of global and local regulation of transcription in DD. We first discovered three *de novo* novel nonsense and frameshift indels in *MED12* in three female patients with Hardikar syndrome - all having cleft palate, congenital heart disease, biliary abnormalities, renal abnormalities, and retinal pigmentation. All three individuals showed extreme skewed X-inactivation (99:1). Our results reveal insights into genetic control of human development.

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P11.035

CIntellectual disability caused by de novo heterozygous mutations in *DYRK1A* gene: first clinically diagnosed patients and a new hotspot mutation

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Introduction: Pathogenic variants in *DYRK1A* resulting in gene haploinsufficiency are responsible for autosomal dominant intellectual disability (ID) type 7 (MRD7, MIM 614104). It accounts for 0.5% of individuals with ID and/or autism. Hitherto 61 molecularly confirmed patients have been published, all identified by exome sequencing without a clinical suspicion.

Clinical cases: we report on three unrelated female patients aged 8, 9 and 5. They all present with microcephaly, ID, autism, stereotypies, seizures and hyperopia. Their clinical phenotype includes deep-set eyes, retrognathia, narrow thorax, slender digits and anterior placement of anus. First patient also has a heart defect and tibial osteochondrosis. Tentative diagnosis of MRD7 was put forward and a known heterozygous variant c.613C>T (p.R205*) was identified in *DYRK1A*. This diagnosis made us think about the second patient, who had been discharged without diagnosis. *DYRK1A* analysis revealed a new variant c.665-5_665-4delTT. First patient´s mother told us about a third girl attending early intervention program with physical resemblance to her daughter. The same variant as in patient 2 was found. All variants occurred de novo.

Discussion: we present the first patients clinically diagnosed with MRD7, mainly characterized by ID, autism, epilepsy, microcephaly and recognizable craniofacial features. Uncommon features such as anteriorly placed anus and tibial osteochondrosis could help to better delineate its phenotype. The identification of the same variant in two unrelated patients points to a new hostpot mutation. Haploinsufficiency of *DYRK1A* results in a clinically distinct and probably underdiagnosed entity, which should be considered in individuals with Angelman syndrome-like syndromes.

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P11.036D

Balanced *de novo* translocation disrupting *EFNA5* in a patient with dysmorphic features, bilaterally cloudy cornea and portosystemic venous shunt

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Primary vitreous regression is a critical event in mammalian eye development required for proper ocular maturity and unhindered vision. Failure of this event results in persistent

hyperplastic primary vitreous (PHPV), also identified as persistent fetal vasculature (PFV). In 2014 Son AI et al. suggested a critical role of ephrin-A5 in regulating proper cell migration into the primary vitreous during early eye morphogenesis in mice. So far there were no reports of studies of *EFNA5* gene in humans. Here we present a boy born with bilateral cloudy cornea and subsequent diagnosis of persistent fetal vasculature, in whom *de novo* balanced translocation disrupted *EFNA5* gene. This 3 years old boy was born with dysmorphic features, hypotonia, bilateral cloudy cornea, heart defect and portosystemic venous shunt, diagnosed as Abernethy malformation type 2. The ophthalmologic evaluation lead to diagnosis of persistent fetal vasculature (PFV). Karyotype in the boy showed a *de novo* translocation 46,XY,t(5:8)(q22q13). Microarray-based comparative genomic hybridization showed normal results. Shallow genome-wide mate-pair library sequencing was applied to identify the area where the break points were present. The PCR product was sequenced with Sanger sequencing. The region containing the break point on chromosome 5 was found to disrupt the 3rd intron of *EFNA5* gene, while on chromosome 8 it was located in non coding DNA. To the extent of our knowledge this is the first report associating *EFNA5* defect with human disease. This study was supported by the NCN Grant No. UMO-2016/21/B/NZ5/02541

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P11.037A

Analysis of *PRRT2* gene : improvement of NGS technology allows us to detect 400% more mutations within homopolymer region as well as CNVs

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The use of multi-gene panels in routine diagnostic of complex pathologies like epileptic encephalopathies is more and more essential. However, using a reliable technology associated with powerful bioinformatics software is highly required to improve diagnostic rate. Here, we compare the use a 150 genes panel (pyrosequencing technology), involved in epileptic encephalopathies and intellectual deficiency (since Oct, 2013) and a second capture based 70 genes panel (SophiaGenetics and Illumina sequencing). With the first panel, *PRRT2* gene had a coverage of 98%, and the most frequent pathogenic mutation in this gene (NM_001256442.1:c.649dup, p.Arg217Profs*8) was not detected because it is included in an homopolymer region of 9 Cytosine. From Oct, 2013 until Dec, 2016 only 3 pathogenic mutations have been identified within *PRRT2* gene by NGS. Since the implementation of the new method in Jan, 2017, 14 additional pathogenic mutations were identified (100% coverage of *PRRT2*) increasing our pathogenic mutations detection rate up to 400%. Then, *PRRT2* mutations represent 1.9% of our cohort. Two types of CNVs have been found, a complete gene deletion and a complete gene duplication. The most known single base duplication (c.649dup, p.Arg217Profs*8) was found 7 times as well as 1 deletion at the same position (c.649del, p.Arg217Glufs*12). A second duplication within an homopolymer of 7 Cytosine was also detected once (c.629dup, p.Ala211Serfs*14). The use of an appropriate technology and a powerful bioinformatics software allowing the simultaneous detection of CNVs, SNVs or InDels in simple region as well as in homopolymer region is highly required for diagnostic purpose.

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P11.038B

A novel mutation in *MYCN* gene causes an unusual presentation of Feingold syndrome

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Background: Feingold syndrome 1 (FS1) (OMIM#164280) is an autosomal dominant malformation syndrome characterized by digital anomalies, microcephaly, facial dysmorphism, gastrointestinal atresia and mild to moderate learning disability. Mutations in *the MYCN* gene (OMIM#164840) are known to cause FS1. Congenital Absence of the Flexor Pollicis Longus (CAFPL) tendon is a rare sporadic hand anomaly. We describe a pedigree with CAFPL, consistent with an autosomal dominant inheritance. Physical and radiographic examinations of family members revealed variable features of Feingold syndrome.

Methods: We performed whole exome sequencing of 5 related patients from the same family and one unaffected married-into the family relative ($n = 6$). Bioinformatic

analysis focused on an autosomal dominant inheritance model.

Results: Whole exome analysis revealed a novel heterozygous mutation (c.1171C>T; p.Arg391Cys) in the *MYCN* gene. Full segregation confirmed the association between the phenotype and the mutation in the family.

Discussion: CAFPL diagnosis should be considered if a patient is unable to flex the interphalangeal joint of the thumb. A hypoplastic thumb or an absent interphalangeal joint crease may be a diagnostic feature. Most cases are sporadic and, to the best of our knowledge, no familial cases have been published so far. Variants in *MYCN* have not been previously associated with CAFPL. This report widens the spectrum of *MYCN*-related disorders suggesting that *MYCN* gene mutations should be included in the differential diagnosis of patients presenting with familial "isolated" CAFPL.

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P11.039C

Contribution of whole exome sequencing in the diagnosis of syndromic developmental abnormalities in fetuses

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Multiple Congenital Anomalies (MCA) are defined by the association of at least 2 congenital malformations. The etiological diagnosis of these conditions is needed for genetic counseling and prenatal or preimplantation diagnosis. The rate of diagnosis for MCA fetuses is about 30% with current diagnostic tests. Most parents are not provided with accurate genetic counseling. We aimed to assess the contribution of whole exome sequencing (WES) to fetal MCA diagnosis after conventional genetic testing. We performed solo WES in 105 fetuses (58 male and 47 female) with MCA and normal array-CGH. Fetal examination did not suggest any clinical diagnosis. The 105 fetuses presented with facial dysmorphism (50%), and intrauterine growth retardation (39%), as well as brain (46%), heart (38%), skeletal (33%), urogenital (27%), digestive (23%), respiratory (21%) or distal limb (15%) anomalies. WES identified a pathogenic variant in 17 fetuses (21%), a variant of unknown significance in 4 fetuses (5%) and a candidate gene in 4 fetuses (5%). This diagnostic yield is lower than in other postnatal MCA cohorts. This result is probably due to extreme, atypical or unspecific phenotypes identified in fetuses, the lack of neurodevelopmental data in this population and the small number of studies performed in fetuses, which limits data sharing. To conclude, the efficacy of solo WES in fetuses is lower than for the postnatal period for MCA. Sporadic variants could be easily identified through trio analysis of the negative cases by second-step parental WES, increasing the rate of candidate variants and the identification of new genes.

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P11.040D

Another case of Galloway-Mowat Syndrome associated with a homozygous mutation of the *OSGEP* gene

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Introduction: Galloway-Mowat syndrome (GWS) is a rare disease entity associating microcephaly and developmental delay with glomerular proteinuria progressing to cortico-resistant nephrotic syndrome and end-stage renal disease.

Six genes are now known to be implicated in GWS including the *OSGEP* gene.

Here, we describe another case of GWS associated with a homozygous missense mutation of the *OSGEP* gene.

Results: At 30 weeks gestation, fetal MRI confirmed the presence of microcephaly associated with bilateral frontal pachygyria in our patient. Proteinuria was detected soon after birth (3g/l). At 6 months, the child presented severe developmental delay, died at the age of 8 months. The index case also presented several dysmorphic features, as previously described associated with KEOPS-complex mutations.

Our index case was the fifth child of consanguineous parents; the parents had had two children born with 'a very small head' who had died in infancy of terminal renal failure.

SNP-array showed multiple regions of homozygosity including the 14q11.2 region. Sequencing of the *OSGEP* gene revealed the presence of a homozygous c.953C>T missense mutation.

Conclusions: Pathogenic mutations of *OSGEP* have been shown to induce defects in the cytoskeleton and to decrease the migration rate of podocytes, thus linking the major manifestations of GWS, structural brain anomalies and nephrotic syndrome.

Hereby, we present another case of nephrotic syndrome with primary microcephaly and brain anomalies, also known as GWS, associated with a homozygous mutatin of the *OSGEP* gene.

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P11.041A

Isolation of endothelial lymphatic cells from patients with generalized lymphatic anomaly

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Introduction: The generalized lymphatic anomaly (GLA) is characterized by lymphatic malformations (LM) and osteolysis. Its genetic cause is unknown, although its pattern of distribution is suspected to be a mechanism of genetic mosaicism, probably limited to lymphatic endothelial cells (LEC).The isolation of LECs from affected tissue of patients affected by GLA has not been described so far, and will allow the detection of possible mutations in mosaic that cause the disease.

Materials and methods: We present a protocol for isolation of LECs derived from LM both sporadically and from a patient with GLA. The fresh tissue is enzymatically digested and the cells are subjected to a Percoll density gradient. The isolated cells are expanded in culture and selected by immunomagnetic methods (MACS), using specific markers of LECs: CD31 and Podoplanin (PDPN). The purified LM-LECs are verified by immunofluorescence with other markers of LECs (VEGFR3, PROX1 and LYVE-1).

Results: The enzymatic digestion of the tissue with collagenase is critical to obtain a high yield and viability of the starting cell population. The Percoll gradient reduces the contamination by non-endothelial cells, which facilitates the subsequent selection by MACs and allows obtaining a population of pure LECs. These results require further phenotypic and functional studies: immunohistochemistry, proliferation and apoptosis assays,, etc.

Conclusions: We have established an isolation protocol and obtained LM-LECs from a patient with GLA. This will allow us to delve into the genetic causes of the disease, as well as perform functional studies that reveal possible targets for new treatments

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P11.042B

Whole Exome Sequencing identifies *WDR47* as a new candidate gene for heterotaxy syndrome

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Introduction: Heterotaxy syndromes represent lateralization defects. The determination of left-right body axis during early embryogenesis depends on a leftward flow generated by rotating primary cilia of the primitive node. Heterotaxy syndromes comprise complex heart defects (CHD), abdominal situs abnormalities including intestinal malrotations, biliary atresia, asplenia, or polysplenia. The birth prevalence is 1/15.000.

Methods: We performed whole exome sequencing (WES) in five parent-child-trios to identify potentially *de novo* or autosomal recessive disease-causing variants. All patients had CHD and a variable heterotaxy phenotype. All parents and siblings underwent thorough ultrasound studies to exclude mild phenotypes. WES filter criteria included: MAF≤0.1%, protein altering, high conservation and deleterious *in silico* prediction (Sift, PolyPhen-2, CADD). Identified variants were validated using Sanger Sequencing.

Results: Filtering of WES data revealed *WDR47* as a novel candidate gene. Here, a *de novo* variant (c.2077G>A, p.Val693Ile; NM_001142550) was identified in the affected child. According to gnomAD browser beta, the variant was found once in 30978 alleles. *WDR47* encodes a protein known to regulate autophagy and microtubule dynamic instability. Moreover, it is involved in developmental disorders of the brain, notably corpus callosum defects.

Discussion: *WDR47* is a promising candidate gene for heterotaxy syndromes due to its central role in microtubule dynamics and function. Screening of the identified candidate gene in larger patient cohorts is warranted. Additional 15 families with heterotaxy syndromes will soon be analyzed using WES and the data will be presented at the conference.

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P11.043C

Unexpected diagnosis in a patient presenting with iris heterochromia and white forelock

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The combination of iris heterochromia and patchy hair depigmentation is strongly suggestive of Waardenburg syndrome, but should not be considered pathognomonic. We report a 2 years old girl who presented at birth with iris heterochromia and patchy depigmentation of hair. Ophthalmological examination also showed unilateral hypopigmentation of the fundus. Her mother reported early greying of hair. On physical examination, the patient also had hypertelorism, epicanthus and left gum and palate hypertrophy. Length was on the 75th centile and head circumference on the 98th centile; she had no other body asymmetries, normal skin and normal development. Genetic testing for the known genes associated with Waardenburg syndrome was negative, and kidney ultrasound, performed because of the gum hemihypertrophy, was normal. A brain MRI showed multiple bilateral subcortical hyperintensities suggestive of Tuberous Sclerosis (TSC) and a bony lesion of the left maxilla, likely due to fibrous dysplasia. Genetic testing for TSC showed a heterozygous *de novo TSC1* pathogenic variant affecting a splice donor site, confirming the diagnosis of TS. White tufts of the hair and, more rarely, patchy area of iris depigmentation, have already been described in patients with TS, but the association of both is not a typical presentation (McWilliam and Stephenson, 1978; Rowley et al., 2001). An early diagnosis of TSC is desirable to allow an appropriate management. This case confirms that the finding of patchy depigmented hair and iris depigmentation should prompt careful skin examination and that TSC should considered as a possible differential diagnosis in these cases.

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P11.044D

A rare case of chromosomal mosaicism with seven cellular lines that contains a jumping translocation that imply the chromosome 14 in a newborn with craniofacial dysmorphia and multiple congenital anomalies

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We present a premature boy born at 35 WA that has a plurimalformative syndrome characterised by:

dolicocephaly, sloping forehead, small nose, micrognathia, abnormal ears (low set and posterior rotated), bilateral criptorhidy, cardiomegaly with persistent ductus arteriosus, cerebral ventriculomegaly and agenesis of corpum calosum. We made GTG banding chromosomal analyse and we discovered a complex mosaicism characterised by the presence of a jumping translocation that imply the long arm of chromosome 14. The chromosomal formula was: 45,X,-14, der(Y)t(14;Y)(q11.2;q12)[76]/45,XY,-14,der(1)t(1;14)(q44; q11.2)[8]/45,XY,-14,der(5)t(5;14)(q15.3;q11.2)[7]/45,XY,-14,der(6)t(6;14)(q27;q11.2)[4]/45,XY,-14,der(21)t(21;14) (q10;q11.2)[3]/45,XY,-14,der(20)t(20;14)(q13.3;q11.2)[1]/ 45,XY,-14,der(22)t(22;14)(q10;q11.2)[1]. We applied also the MLPA with telomere probes (P-036 kit and P-070 kit, MrcHolland®) and we discovered a deletion of approximate 2,21Mb located on chromosome 14. Using the P358 kit (MrcHolland®) we confirmed the absence of segment between the position 19,863,569 and 22,127,740 on chromosome 14. Jumping translocations are extremely rare and represents the translocation of the same chromosomal fragment to different other chromosomes in different cell lines. The majority of cases were described in different type of cancers, but also jumping translocations were found in constitutional cytogenetic associated with an abnormal pattern of development. The breakpoints implied in jumping translocations are located in chromosomal regions that contain repetitive DNA. This feature is present also in our case, when the breakpoint on chromosome 14 is on the long arm in proximity of centromere while the rest of breakpoints imply the telomeric region. In conclusion, we presume that in our case the congenital anomalies were generated by the absence of a small segment of chromosome 14 produced during the complex mechanism of jumping translocation.

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P11.045A

Kabuki syndrome and immune manifestations: a cohort of 176 patients

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Introduction: The kabuki syndrome (KS) (MIM 3147920 and 300867) is a rare malformative syndrome, including specific facial features, moderate to severe intellectual disability and various malformations. A high prevalence of immunological manifestations is observed in Kabuki patients. Immune function impairment reduces the prognosis whereas this aspect is one of the less studied in the literature. To prove the importance of the management those manifestations, we measured the prevalence of immune manifestations. We analyzed data to detect particularities of presentation, phenotypic associations and therapeutic effectiveness on a large cohort.

Methods: The 176 Kabuki patients included were followed by 30 French centers and molecularly confirmed. *(KDM6A* or *KMT2D*). Questionnaires assess the presence of immune deficit and autoimmune diseases and on clinic and biological basis.

Results: 44,6% of our patients had repeated infections (mainly ENT) and 61,6% had hypogammaglobinemia. 13.2% patients had an autoimmune disease and 5% had two or more. Prevalence in adult patients raised to 25,8%. The most frequent autoimmune manifestation is immune thrombocytopenic purpura (8,6%, RR: 215). Autoimmune hemolytic anemia is observed in 4,0% (RR: 358). Among

non-hematological manifestations, vitiligo and autoimmune thyroiditis were frequents. Immune deficiency during childhood is correlated with susceptibility to autoimmunity in adults

Conclusion: We measure a high prevalence of immune manifestations, demonstrating the importance of an efficient management of this frequent, treatable and sometimes severe aspect of KS whereas during the time of our study, data where incomplete. This phenomenon could be explained because Kabuki genes are indirectly involved in B and Treg lymphocytes differentiation.

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P11.046B

Mosaic mutation in *KDM6B* gene in patient with Kabuki syndrome: analysis of genotype-phenotype correlation

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Introduction: Kabuki syndrome (KS) is a rare disorder characterized by distinctive face, congenital anomalies and intellectual disability caused by mutations in *KMT2D* and *KDM6A*, two interacting chromatin modifiers responsible for 56-75% and 5-8% of KS, respectively. To date, only six KS patients with mosaic *KMT2D* mutations were described. Any mosaicism in *KDM6A* have been reported so far.

Methods and Results: A 2.5-years-old male is the first child of healthy non-consanguineous parents with negative family history. At the age of 1 year he had his first medical examination because of hypoglycemia and muscular hypotonia. A diagnosis of glycogen storage disease type 0 or fructose-1,6-bisphosphatase deficiency were suggested, but the mutational analysis using customized TruSight One panel did not confirm it. Nevertheless, NGS revealed a novel frameshift deletion, c.4160_4161del/p.Tyr1387*, in approximately 65% of X-linked *KDM6A* alleles derived from the patient's leukocytes. This result might indicate a diagnosis of KS, that has been finally concluded after analysis of genotype-phenotype correlation. Characteristic facial cue, abnormalities of growth and development, as well skeletal anomalies seen in the presented patient were consistent with clinical expression of KS.

Conclusions: This is the first report of a patient with mosaic mutation in *KDM6A* and clinical features of KS. His KS phenotype is not consistent with previously reported in *KMT2D*-positive patients with mosaicism, who had only mild facial dysmorphism. Genotype-first approach combined with reverse phenotyping has shown to be a powerful tool in human genetics, especially in the era of next-generation sequencing. Study was supported by CMHI project S149/16.

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Improve Kagami-Ogata syndrome understanding through induced pluripotent stem cells from two patients with a deletion not including the imprinting centers

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Imprinting disorders are associated with the alteration of genes differentially expressed between maternal- and paternal-inherited chromosomes, as a consequence of abnormal methylation pattern in regulatory elements called imprinting centers (ICs). Kagami-Ogata syndrome (KOS) is a rare and sometime lethal congenital disorder caused by alteration at the 14q32 maternal region, between *DLK1-DIO3*. The region hosts two long-RNAs, *MEG3* and *MEG8*,

and clusters of small-RNAs. Although a few cases of KOS have been reported with partial deletion of the region, the specific role of its non-coding genes within the pathogenesis of KOS has not been yet clarified.

To improve the understanding of KOS mechanisms, we generated iPSCs from KOS sibship having a 130Kb deletion at 14q32.2, which interrupted *MEG3* and eliminated several non-coding RNAs, inherited by the healthy mother. We used a non-integrative system, to not alter patients' genotype.

Mature iPSCs were characterized by Sanger sequencing, qPCR and karyotyping to confirm rearrangement stability and immune-fluorescence to test iPSCs quality. We succeeded to obtain patient-derived iPSCs showing stem cells features such as expression of specific markers and pluripotency. Further analysis revealed that the methylation status was not altered.

We created the first human model of KOS maintaining patient's genotype and methylation status. Furthermore, our model allows to reveal a correlation between phenotype and individual genes in 14q32 domain because our patients' rearrangement does not alter ICs functionality. Also, iPSCs we generated allow to study the rearrangement in adult tissues by direct differentiation in culture and rescuing the deleted genes through gene editing.

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Molecular deepening by *ANKRD11* gene expression analyses of KBG patients harboring submicroscopic rearrangements

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KBG syndrome (KBGS) is a disorder characterized by short stature, distinctive facial features and developmental/cognitive delay, caused by *ANKRD11* gene mutations/deletions at 16q24.

Here we describe the utility of *ANKRD11* RT-qPCR gene expression analysis to investigate the effect, at transcript level, of *ANKRD11* sub-microscopic rearrangements in four patients with KBGS/KBGS-like clinical diagnosis.

RT-qPCR in the first patient, with a *de novo* deletion involving the last two *ANKRD11* exons, confirmed the molecular defect and identified not only a halved amount of wt transcript, which is indicative for KBG diagnosis, but also an aberrant mRNA in the expected size, likely truncated and dysfunctional.

RT-qPCR proved of great clinical utility in evaluating the pathogenic effect of a partial *ANKRD11* duplication in a patient with a less convincing KBGS phenotype, as it revealed mRNA levels comparable to controls.

ANKRD11 haploinsufficiency was again confirmed by RT-qPCR in two further patients: the first with a known *de novo* molecular defect in IVS1 encompassing part of the *ANKRD11* promoter region and concordant clinical features; and the second with a clear KBG clinical diagnosis but negative molecular investigations. Consistent with the RT-qPCR data further molecular characterizations were performed in the second patient and a 1.8 kb deletion involving the gene promoter was identified.

This preliminary data proved RT-qPCR is a helpful tool for molecular and clinical diagnosis both in patients with *ANKRD11* sub-microscopic rearrangements, in which molecular effect is uncertain, and in molecularly unsolved KBGS patients.

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Macrophthalmia and large vessels aneurysms: a coincidence?

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Dilatation of large thoracic vessels, either in its isolated or syndromic form, is highly heterogeneous, with disease-causing mutations in over 30 genes being identified so far. However, none of the associated syndromes presents with macrophthalmia. We report on a 31-year-old female with a history of HLAB27-related bilateral anterior uveitis in the context of severe and rapidly progressive myopia occurring at 6 years of age. Exophthalmia was observed in the acute phase and progressed regularly thereafter, leading to retinal detachments and painful disproportionate enlargement of eyeballs (transverse diameter > 32mm, NR mean 24,5mm). At 12 years, a routine heart ultrasound detected an aortic dilatation (aortic root +3 SD, ascending aorta +7 SD), also progressive, prompting funnel replacement of aortic ascending aorta at 18 years. Dilatation of the brachiocephalic arterial trunk and bilateral carotid dysplasia are observed, raising the hypothesis of a connective tissue disorder. Blindness in the left eye with phtisis bulbum occurred at 27 years. Transient ischemic attacks occurred at 29 years manifesting by focal neurologic deficits. A 31 genes TAAD panel did not identify any mutation. A trio WES yielded two relevant **Results:** a *de novo CCDC51* c.634C>T(p.R212X) variant and a *BCORL1* compound heterozygosity c.3158A>G(p.K1530R) and c.185T>C(p.L62P). No disease-causing mutation in *BCORL1* has been identified so far. We suggest that this singular association of progressive megalophthalmia and dilatation of the great vessels is not coincidental and represents a new entity awaiting description of additional cases to be confirmed. Functional studies are underway to confirm this hypothesis.

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Truncating variants in the paternally expressed allele of *MAGEL2* as a common cause of syndromic distal arthrogryposis

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Introduction: We describe four individuals with truncating variants in the paternally expressed allele of the, maternally imprinted *MAGEL2* gene, responsible for Schaaf-Yang syndrome (SYS). Patients suffering from SYS present with hypotonia, global developmental delay (DD)/intellectual disability (ID) and feeding difficulties. Additional features include higher prevalence of autism spectrum disorder (ASD), joint contractures, sleep apnea and lowered bone density.

Materials and methods: - Variants were identified by whole exome sequencing or using a NGS custom panel containing a set of genes involved in ID, ASD and other common genetic conditions. - Sanger sequencing was performed to determine whether the variant was de novo or inherited. - Methylation-sensitive digestion followed by PCR amplification was used to ascertain the parental origin of the variants.

Results: *MAGEL2* variants were found on the paternal allele in all four subjects. Two of them were paternally inherited while the other two were de novo. A detailed clinical description of these individuals and a review of previously reported cases will be provided.

Conclusions: *MAGEL2* truncating variants are a hitherto unrecognized likely "common" cause of syndromic distal arthrogryposis but the exact proportion of cases explained by this gene still needs to be ascertained. We suggest that a specific analysis pipeline that does not include inheritance filtering should be used for the analysis of imprinted genes such as *MAGEL2*.This work was supported by a grant of the Spanish Institute of Health Carlos III, ISCIII (PI13/02010).

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P11.051C

Melorheostosis and vascular anomalies associated with *KRAS* mosaicism

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Introduction: Melorheostosis is a rare sclerosing bone dysplasia resembling dripping candle-wax along bones on radiographs and usually follows a sclerotomal distribution. Often neighbouring extraosseous anomalies are associated, i.e. scleroderma-like skin changes. We present a case of polyostotic melorheostosis with multiple vascular (arterial, lymphatic) anomalies and a hyperpigmented patch.

Case description: A 12 year old girl was referred for assessment. Family and prenatal history were unremarkable. She was born preterm with normal birthweight. She had congenital chylothorax and aortic coarctation. Diffuse pulmonary lymphangiomatosis caused a moderate restrictive lung disease. Stenosis of other arteries (superior mesenteric artery, celiac trunk and right renal artery, causing hypertension) were detected. Sclerosing bone changes became apparent and affected only the upper left side of her body (from metacarpal bones to scapula, extending to ribs and vertebral bodies, causing scoliosis), characteristic of melorheostosis. She had normal growth parameters and no learning difficulties. On examination, upper limb and rib cage asymmetry were evident.Also a large hyperpigmented patch with geographic borders on her trunk, and a reddened soft plaque at the lower neck (lymphatic malformation on biopsy).

Results: NGS of the cervical lymphatic malformation and the hyperpigmented patch revealed mosaicism for a heterozygous *KRAS* mutation (Q61H): 40% and 4%, respectively. The mutation was absent in blood leukocytes.

Conclusions: The same *KRAS* mutation was detected in another case of melorheostosis with no vascular anomalies. Our case contributes to the hypothesis of a postzygotic mosaicism as the disease causing mechanism of melorheostosis and widens the clinical spectrum of mosaic RASopathies.

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P11.052D

A microcephalic osteodysplastic primordial dwarfism type 2 case with a homozygous novel mutation in *PCNT*

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Introduction: Microcephalic Osteodysplastic Primordial Dwarfism Type 2 (MOPD2) is a rare autosomal recessive syndrome with extreme prenatal and postnatal growth retardation, microcephaly, characteristic facial appearance and skeletal findings, caused by biallelic mutations in the Pericentrin gene (*PCNT*). We report a case of MOPD2 with a novel mutation in *PCNT*.

Materials and methods: Targeted next-generation sequencing (NGS) including *PCNT* gene was performed in the case with the clinical diagnosis of MOPD2 at the Institute of Genetics and Molecular Medicine, in Edinburgh University.

Results: A 17-year-old male who had a history of repetitive afebrile seizures, was consulted to the genetics department while being interned at the emergency ward due to respiratory distress, confusion, and hypertension. The case had severe growth retardation and microcephaly, with a height of 98 cm [-11,35 SD], and OFC of 38 cm [-11,64 SD]. Dysmorphic features included a prominent nose, micrognathia, microdontia, sparse hair, hyper-hypopigmented skin lesions and clinodactyly. MRI angiography showed a "cigarette smoke" view that is suggestive of Moyamoya disease. X-rays showed small iliac wings and coxa vara. Targeted NGS analysis of *PCNT* revealed a novel homozygous variant, c.3465-1G>A, in the patient. Parents were heterozygous for the same variation. It is predicted to be disease causing by disrupting the splice region of the exon 18.

Conclusion: MOPD2 significantly overlaps with the Primary autosomal recessive microcephaly/Seckel syndrome spectrum, but definitive diagnosis is important regarding follow-up of vascular central neural system complications, such as Moyamoya disease. Identification of the disease-causing mutation is important for early prenatal diagnosis.

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P11.053A

Growth pattern and morphologicalcharacteristics of the fingers in Mowat-Wilson syndrome

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Mowat-Wilson syndrome (MOWS) is caused by de novo heterozygous loss of function mutations or deletions of the ZEB2 gene. Patients present with mental retardation, epilepsy, and characteristic facies. Health care for genetic syndromes requires data on standard growth patterns; however, data on patients with MOWS have not been documented in detail. We report growth patterns and other physical characteristics of patients with MOWS. This study collected physical measurements of patients with MOWS and examined the finger morphology. Our results showed that the physical findings characteristic of this syndrome included measurements at birth showing values within the normal range, with subsequent postnatal growth impairment, microcephaly and thin habitus. Fingers in patients with MOWS are bamboo-like, long and thin, with prominent joints. The skin is thin, mildly redundant, and hyperextensible. Combined with the typical facial characteristics of MOWS, these findings could be a clue to phenotypic diagnosis in this syndrome.

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P11.054B

Report of a case with multiple joint dislocation syndrome associated with a homozygous pathogenic mutation in the *EXOC6B* gene

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Introduction: Spondyloepimetaphyseal dysplasia (SEMD) is an inherited disorder characterised by joint dislocation at birth, thin limbs, joint laxity, poor bone classification and delayed bone age. Skeletal dysplasia with multiple joint dislocation are various group of disorders comprising differential diagnosis.

Case: A 21 month old boy with multiple joint dislocations joint laxity, born to consanguineous parents was referred to our clinic of genetic. Patient suffered from hip, knee and elbow dislocation, joint slackness, kyphoscoliosis, sever laxity of wrist joints, delayed bone age, flat feet and cryptorchidism.

Material & methods: Whole Exome Sequencing were used to enrich all exons of protein-coding genes as well as some important other genomic regions. Next generation sequencing was performed to sequence close to 100 million reads on Illumina Sequencer. Bioinformatics analysis of the sequencing results was performed using international databases and standard bioinformatics software. This mutation was confirmed in proband and parents by sanger sequencing.

Result: Whole Exome sequencing identified a novel stopgain homozygous variant c.C958T p.R320X in exon 9 of the *EXOC6B* gene. The variant was assessed by analytical software and multiple databases. Genotype-Phenotype correlation and co-segregation analysis was confirmed among family members to confirm the pathogenicity of the alteration.

Discussion: Here we report for the second time a novel mutation in the *EXOC6B* gene known to cause this different type of skeletal dysplasia in an Iranian patient. Till date only one mutation was reported in this gene (Girish *et al*.,2016).

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P11.055C

Analysis of mutational load in Joubert syndrome genes in affected individuals compared to controls

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Next-generation sequencing frequently uncovers multiple rare, predicted-deleterious variants (RDVs) in different genes associated with the same recessive disorder in any individual, described as "mutational load". While such RDVs could contribute to "oligogenic inheritance" or act as "genetic modifiers", their clinical significance remains unclear. Focusing on the genetically highly heterogeneous recessive ciliopathy Joubert syndrome (JBTS), we undertook a systematic analysis of RDVs in 25 JBTS genes, comparing ~400 affected individuals with JBTS to in-house controls and to UK1958 birth-cohort per-sample exome data. Using criteria for deleteriousness established in the JBTS cohort, we identified a surprisingly large number of controls harboring RDVs in JBTS genes (~30%), whereby the type/distribution of variants in controls differed substantially from the causal alleles in affected individuals, suggesting that despite the predictions, the majority of variants in controls are not disease-causing. RDVs in ≥2

genes were common in affected individuals *and* in controls, as predicted by allele frequencies in ExAC (probability of heterozygous RDVs in any 2 of 25 genes =~9%). 38% of affected individuals carried bi-allelic causal variants in one gene plus additional RDVs in other gene(s). Phenotypic discordance was observed between 60% of affected individuals sharing identical causal alleles, supporting existence of genetic modifiers. However, the presence of RDVs in addition to causal variants did not correlate with phenotypic severity, indicating that simple addition of RDV numbers has no predictive value. While interpretation of the phenotypic effect of RDVs remains challenging, comparison of variant types/distribution between causal alleles and control/additional alleles can provide valuable insights.

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P11.056D

The co-existence of Nablus Mask-Like Facial Syndrome and Klinefelter Syndrome

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Introduction: Nablus mask-like facial syndrome (NMLFS) is a rare microdeletion syndrome. A mask-like facial appearance is the characterized symptom of disease. Here we report the co-existence of NMLFS together with Klinefelter Syndrome in a patient, for the first time in the literature, to the best of our knowledge.

Clinical Report: A five years old male patient was referred to our clinic because of speech delay, growth retardation, mental retardation and dysmorphic features. He was a born at 38 weeks gestational age to non-consanguineous parents. His weight, height and head circumference percentiles were <3p at the time of physical examination. Microcephaly, hyperthelorism, upper epicanthus, wide nasal base, high arched plate, micrognatia, protruding ears and hypomimic, mask-like face were the other physical examination findings. His electrocardiogram, hearing test, abdominal USG and cranial MR were normal and Denver II developmental screening test showed development delay. The patient's karyotype was 47,XXY, compatible with Klinefelter syndrome. Since this karyotype was not enough to explain the patient's dysmorphic features and motor and mental retardation we performed microarray analysis. The microarray analyses revealed a 5,024 Kb deletion on 8q21.3q22.1 which contains 17 OMIM genes. This deleted region is the region associated with NMLF Syndrome and explains our patient's clinical findings. Parental karyotypes were normal.

Conclusions: Nablus mask-like facial syndrome is a rare microdeletion syndrome. According to the literature, this is the first time that NMLFS and Klinefelter's Syndrome are together in a patient.

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P11.057A

NBAS associated disease: further defining the phenotype of a recognizable syndrome

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Introduction: Biallelic pathogenic variants in the *NBAS* gene are associated with two different phenotypes: infantile liver failure syndrome 2 (OMIM 616483) and short stature, optic nerve atrophy, and Pelger-Huet anomaly (SOPH) syndrome (OMIM 614800).

Patients and Methods: two unrelated patients, a 4-year-old female and a 7-year-old male, were referred to our Genetics Unit. They presented with a highly overlapping phenotype, characterized by prenatal hyposomatism evolved into harmonic short stature, persistent hypertransaminasemia, hypogammaglobulinemia and hypovision. The second patient also presented congenital glaucoma and primary hypothyroidism. They both presented slight psychomotor delay and mild dysmorphic facial features with hypotelorism, smooth philtrum and narrow mouth. Both patients were enrolled in the Undiagnosed Patients Program of the Ospedale Pediatrico Bambino Gesù. Whole exome sequencing and cDNA analysis were performed on probands' genomic DNA and RNA extracted from PBMC, respectively.

Results: Both patients carried a nonsense mutation in *NBAS* (NM_015909.3; p.Arg501* and p.Ser230fs*4) in compound heterozygosity with the synonymous variant c.6840G>A (p.Thr2280Thr). This variant (MAF< 0.00001, ExAC) affects the last nucleotide of exon 51, and cDNA

analysis demonstrated its pathogenicity, causing skipping of the exon.

Conclusion: The presence of biallelic inactivating variants in *NBAS* in the presently reported cases prompts the unification of dependent infantile liver failure syndrome 2 and SOPH syndrome in a single autosomal recessive condition characterized by variable association of hypertransaminasemia, recurrent acute liver failure, short stature, bone fragility, ocular defects and immune system disorders.

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P11.058B

Trio based exome analysis results at the Spanish Undiagnosed Rare Diseases Program, SpainUDP

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Introduction: SpainUDP is the Spanish Undiagnosed Rare Diseases Program, implemented by the Institute of Rare Diseases Research (IIER) of the Institute of Health Carlos III (ISCIII). Since 2015 works in collaboration with Hospital Puerta de Hierro (Madrid) which supports detailed clinical examination and complementary studies of patients. SpainUDP aims to find a definitive diagnosis to patients with undiagnosed rare diseases, through a multidisciplinary approach (by clinicians, geneticists, bioinformaticians and researchers).

Materials and methods: Taking advantage of next generation sequencing techniques, mainly whole exome analysis (WES) is performed by trio analysis. In addition, Phenotips software is used for an accurate and standardized description of phenotypes (through HPO, Human Phenotype Ontology).

Results: In 2015-2017, 135 cases were accepted in SpainUDP. During this time, 37 cases (27.4%) dropped out the program due to diverse reasons. The remaining 98 cases are distributed as follows: 40 cases are in a deep phenotypic characterization; WES is ongoing for 33 cases; 7 cases are pending on variants validation by Sanger; and phenotypic and genotypic (WES) characterization is finished in 18 cases, of which 13 (72.2%) have been diagnosed. In 77% of diagnosed cases the causal variant corresponded to a '*de novo*' mutation (frameshift and stopgain variants).

Conclusions: For the still undiagnosed cases, functional studies are expected. Moreover, SpainUDP participates in international initiatives such as the European projects RD-Connect and Solve RD, the Undiagnosed Diseases Network International (UDNI), and the MatchMaker Exchange platform, sharing phenotypic and genotypic data to find cases with similar profiles to get a diagnosis.

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P11.059C

Whole exome sequencing analysis candidates *MRVI1* as a potential susceptibility gene for Moyamoya syndrome and cerebral arteriopathies in Neurofibromatosis type 1

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Introduction: Moyamoya disease (MMD) is a progressive cerebral vasculopathy. The p.(Arg4810Lys) substitution in *RNF213* is linked to MMD in Asians. Recently several rare variants in *RNF213* have been associated to MMD in europeans. People with neurofibromatosis type 1 (NF1) are particularly prone to develope this angiopathy thus called syndrome (MMS). Intriguingly, most cases of NF1-related MMS have been described in Caucasians, inverting the population ratio for MMD observed in Asians. Additive

genetic factors, independent of the *NF1* locus, may contribute to the pathogenesis of MMS.

Methods: We carried out a whole exome study in a large Italian family with MMS-NF1 co-occurrence in two first cousins and minor cerebral vasculopathies in NF1 relatives.

Results: None of the *RNF213* variants already reported or other rare variants were found yet the p.(Pro186Ser) substitution (rs35857561) in *MRVI1* segregated with MMS and other minor cerebral vasculopathies in our Italian family.

Conclusions: *MRVI1* is a functional partner of ITPR*1*, *PRKG1* and *GUCY1A3*, differently related to MMS and other vasculopathies, all involved in response to NO. The rs35857561 substitution has got a higher MAF in Europeans than in Asians. The variant segregated with two more patients with MMS form a further NF1 German family. The 11p15.4 cytoband, where *MRVI1* is located, has been linked to retinal vessel diameter, with the *D11S1999* marker closely located to the 5'UTR of *MRVI1*. These reasons support the hypothesis that *MRVI*, and the p.(Pro186Ser) substitution, might really represent a susceptibility factor for MMS in Caucasians with NF1.

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P11.060D

The molecular profile of Polish patients with neurofibromatosis type 1 and neurofibromatosis-Noonan syndrome: do additional genetic factors affect the phenotypic variability among *NF1*-positive patients?

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Introduction: Neurofibromatosis type 1 (NF1) is a RASopathy characterized by neuro-cutaneous abnormalities and predisposition to tumorigenesis. Despite molecular homogeneity (*NF1* gene mutations), significant phenotypic variability is observed, not only among unrelated NF1 patients, but also within affected families. Consequently, a possible correlation of NF1 clinical spectrum with additional genetic factors ("modifiers") has been inferred.

Patients and Results: We present the molecular profile of 45 unrelated Polish NF1 and neurofibromatosis-Noonan patients (and 26 affected family members), expanded by analysis of potential modifiers within other nuclear genes and mtDNA. Using MLPA and NGS sequencing we identified 40 different pathogenic/probably pathogenic *NF1* variants (including whole-gene deletions/duplications), nearly half of them novel. Mutation detection rate in the entire study cohort was 92%, familial cases constituted 56%. To evaluate the potential influence of molecular modifiers, clinical exomes of 37 *NF1*-positive probands were analysed, revealing variants in other clinically relevant genes (oncologic, cardiac, neurologic, cutaneous or immune), including DNA repair-related genes. Co-occurrence of another Ras/MAPK gene variant and concomitance of Silver-Russell syndrome were noted in 6 and 1 patients, respectively. Mitochondrial DNA analysis in 59 NF1 cases excluded known mutations, but did show several secondary/synergistic/modifier alterations or risk factors for neurological, cardiovascular, metabolic disorders or cancerogenesis.

Conclusions: Our study contributes to further delineation of the NF1 molecular profile and presents new data concerning potential factors influencing clinical variability of the disease. More detailed correlation/differentiation studies for *NF1*-related phenotypes may lead to an update of the diagnostic criteria and future therapeutics.

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P11.061A

Neurofibromatosis-Noonan syndrome diagnosed in an infant of a three-generation NF1 family

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Neurofibromatosis-Noonan syndrome (NFNS, MIM: 601321) is a peculiar entity characterized by the clinical signs of both Neurofibromatosis type 1 (NF1) and Noonan syndrome (NS). The features of Noonan syndrome mainly facial dysmorphism and short stature accompany the characteristics of NF1 as café-au-lait spots and skeletal changes.

Neurofibromas and Lisch nodules are less frequently observed in this NF1 variant. In most of the cases published until now a heterozygous mutation in NF1 gene was determined. It was also demonstrated that RAS-MAPK pathway is affected and neurofibromin is a negative regulator of this signal transduction pathway. Therefore, NFNS belongs to the so called RASopathies with Noonan and Noonan-like syndromes. Classical mutations causing NF1 phenotype can also cause NFNS.

We present a three-generation family with the clinical and genetic diagnosis of NF1 following autosomal dominant inheritance (all affected family members meet the NIH-consensus criteria for NF1). The proband is a 6-months-old boy with multiple café-au-lait spots, failure to thrive, relative macrocephaly and facial minor anomalies resembling Noonan syndrome. Mutation was determined in the family to be c.499_502del4bp in heterozygous form. As far as we know this mutation was not published previously in connection with NFNS.

The origin of this interesting phenotype is still debated, the most accepted concept keeps it an NF1 variant and a recent publication suggests that fetal environmental factors may also play a role in the evolvement. We discuss in our poster the current knowledge about the pathogenesis of RASopathies and focus on the unanswered questions regarding NFNS.

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P11.062B

Two brothers with Malan syndrome and identical seemingly de novo missense NFIX variant due to probable paternal germline mosaicism identified using exome sequencing

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Overgrowth syndromes combine height greater than two SDs above the mean and other features. The best recognised is Sotos syndrome (MIM117550), an autosomal dominant disorder with distinctive facial features, intellectual disability (ID), body overgrowth in early life and macrocephaly. Mutations in *NSD1* are found in 90% of cases. *De novo* mutations in the initial exons of *NFIX* in 19p13 have also been identified in 17 patients referred to as Sotos 2 or Malan syndrome (MS, MIM614753).

We present two brothers aged 23 and 7 years with ID, craniofacial dysmorphism (macrodolichocephaly, high forehead and anterior hairline, long face, downslanting palpebral fissures, low-set dysplastic ears, prominent chin) and musculoskeletal, ocular and CNS abnormalities. Exome sequencing identified an identical *de novo NFIX* exon 2 variant NM_001271043.2:c.370C>T p.(Arg124Trp) in both brothers. The variant was also in 1/95 reads in the father. This could be an error or contamination, but it may indicate mosaicism (including germline mosaicism). Paternal origin of the variant was supported also by haplotype analysis: the brothers carried different copies of maternal 19p13 but shared a paternal haplotype. The variant was predicted to be deleterious and was absent from all databases (ESP, ExAC, gnomAD, GEEVS). A substitution affecting the neighbouring residue, p.(Arg123Trp), has been reported in one MS patient.

These two new cases of MS help to define the clinical picture of MS and underscore the recent notion that a significant fraction (4-10%) of *de novo* mutations can originate from germline mosaics, with consequences for recurrence risks.

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P11.063C

Panel based next generation sequencing: pathogenicity assessment of novel variants and their impact in genetic counseling

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Introduction: Identification of disease-causing mutations has been tremendously accelerated by Next Generation Sequencing (NGS) implementation. However, the benefits offered by NGS come with a number of challenges. Novel variants` pathogenicity assignment is one of the biggest, but

in some cases, it might be clarify by a detailed clinical history and a multidisciplinary approach.

Aim: Determine the pathogenicity of novel variants in order to give the correct genetic counseling to families.

Materials and Methods: 80 non related cases were analyzed using NGS panels. Detailed clinical data and genealogies were noted. Written informed consent was obtained from all families before testing. The NGS capture panels were designed and validated by Sistemas Genómicos with CE marking certificate for diagnosis, and performed with Illumina technology. The variants were analyzed using GeneSystems software. Families were assessed and advised by clinical geneticists.

Results: Novel likely pathogenic variants were found in 5 families, and confirmed by Sanger sequencing.

The table describes the variants found in patients, and their clinical diagnosis.

Case	Panel	Novel Variation	Gene	Clinical diagnosis	zigocity
1	Intellectual disability	NM_004456.4: c.2030A>G p.Asp677Gly	*EZH2*	Weaver Syndrome, (autosomal dominant)	heterozygous
2	Neurology	NM_014946.3: c.165C>A p.Tyr55*	*SPAST*	Spastic Paraplegia 4, (autosomal dominant)	heterozygous
3	Cardiology	NM_000116.4: c.202A>T p.Asn68Tyr	*TAZ*	Left ventricular noncompaction, (X linked recessive)	hemizygous
4	Epilepsy	NM_001165963.1: c.2665G>A p.Ala889Thr NM_000068.3: c.6739C>T p.Arg2247Trp	*SCN1A* *CACNA1A*	Epilepsy with Ataxia, (autosomal dominant)	heterozygous
5	Skeletal dysplasia	NM_001287.5: c.2229dupC p. Ser744LeufsTer183	*CLCN7*	Osteopetrosis, (autosomal dominant/ recessive)	homozygous (Allele deletion was ruled out by aCGH)

After deep molecular, clinical and genealogy analysis, further complementary tests in relatives were performed. These results allowed the assessment of pathogenicity of all novel variants. Genetic counseling of families will be discussed.

Conclusion: A detailed clinical history in addition to the panel-based NGS technology, might allow the determination of the pathogenicity of novel variants, improving the genetic counseling of the families involved.

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P11.064D

Application of panel next generation sequencing in the diagnosis and clinical differentiation of patients with Noonan syndrome clinical suspicion

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Introduction: The implementation of the next generation sequencing (NGS) technique allowed not only to identify novel genes related to disease etiology, but also significantly improved molecular diagnosis of Noonan syndrome (NS) and related disorders. The aim of the study was the identification of pathogenic variants in novel and candidate genes related to NS syndrome pathogenesis.

Materials and Methods: One hundred twenty-eight patients with Noonan syndrome clinical diagnosis and excluded mutations in *PTPN11*, *RAF1*, *SOS1* and *KRAS* genes were tested with custom designed NGS panel (SeqCap EZ Choice Library, Roche Diagnostics). Functional *in silico* and *in vitro* (when possible) studies of new mutations were performed.

Results: Panel testing allowed to identify pathogenic / potentially pathogenic variants in 67 (52.3%) probands. Thirty of them had mutation in known RASopathies-related genes including: *NF1* (10pts, all patients besides facial

dysmorphy and other NS related symptoms presented CAL spots), *BRAF* (6pts), *RIT1* (4pts, including twin sisters suspected for NF1/NFNS), *CBL* (4pts) and *SHOC2* (2pts, c.4A>G variant). The NGS panel also included genes related to disorders clinically similar to NS and pathogenic/potentially pathogenic variants in these genes (particularly *KMT2D*, *ARDI1A*, *ARID1B*, *CREBBP*, *KAT6B*) were found in 24 patients. Also, variants in *RASA2*, *MAP3K8*, *LZTR1* or *A2ML1* genes were found in single patients. No variants in *SOS2* or *PPP1CB* were found.

Conclusions: The implementation of panel testing is beneficial in the diagnosis of Noonan syndrome and other RASopathies, although it seems that mutations in "novel" genes are incidental.

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P11.065A
Ten candidate genes sequenced in patients with oculo-auriculo-vertebral spectrum

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Oculo-auriculo-vertebral spectrum (OAVS) is a craniofacial developmental disorder that mainly affects the structures derived from the first and second pharyngeal arches. The phenotype is heterogeneous and typically characterized by abnormal mandibular, oral, and ear development. The spectrum's etiology is complex and heterogeneous since genetic, epigenetic and environmental factors seem to be involved; however, the mechanisms are still not clear. Structure variations have been described as potentially pathogenic for the disorder, but evidences of single nucleotide variants (SNVs) on specific genes are still scarce. So far, *MYT1* gene has been the only gene implicated in some patients with OAVS. Therefore, the investigation of single nucleotide variants (SNVs) on more candidate genes is crucial to understanding this complex disorder. We investigated the coding and UTR regions of ten candidate genes that may be relevant to OAVS: *CRKL, YPEL1, MAPK1, NKX3-2, HMX1, MYT1, OTX2, GSC, PUF60*, and *HOXA2*, by Ion PGM System for Next-Generation Sequencing (Thermo Fisher Scientific). In 89 individuals studied (77 patients and 12 relatives), we identified a total of 194 variants in DNA extracted from peripheral blood. In order to infer the potential pathogenicity of these variants, in silico analysis was performed using the prediction tools Mutation Taster, FATHMM, PolyPhen 2, and SIFT, as well as clinical databases such as ClinVar. Using these approaches, seven SNVs, found in genes *YPEL1, MAPK1, CRKL, OTX2*, and *MYT1*, were considered potentially pathogenic. Also, 44 SNVs with unknown significance were found. Our data confirms the possible genetic heterogeneity in OAVS. Financial Support: FAPESP 2016/18781-7.

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P11.067C
Three unrelated Lithuanian cases of oculodentodigital dysplasia: phenotypic analysis and comparison to the literature

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Introduction: Oculodentodigital dysplasia (ODDD) is a rare autosomal dominant syndrome, caused by a heterozygous mutation in the *GJA1* gene on chromosome 6q22. Around 300 cases have been described in the scientific literature. Features of ODDD include facial, skeletal, neurological, cardiac, and ocular anomalies with high penetrance, intra- and interfamilial phenotypic variability, and advanced paternal age in sporadic cases.

Materials and Methods: Three unrelated Lithuanian patients with genetically confirmed ODDD are reported. A girl, now aged 10, a boy, now aged 9, and a girl, now aged 2, with typical features of ODDD presented bilateral epicanthus, prominent columella, hypoplastic alae nasi, and enamel hypoplasia. Sanger sequencing of coding regions of the *GJA1* gene was performed for all cases. All three identified variants were analyzed with previously reported ODDD phenotypes.

Results: Molecular genetic analysis of the coding sequences of *GJA1* (NM_000165.4, NP_000156.1)

identified such pathogenic variants c.412G>A (p.G138S) in the first (CM086823), c.75G>T (p.W25C) in the second (CM120084), and c.338T>C (p.L113P) in the third case (CM040074). Facial phenotype was consistent throughout the cases. In the literature these variants were associated with neurological anomalies, whereas in our cases such phenotype was not observed. Ocular findings as described for p.L113P, and p.W25C were only present in our case 2. Cardiac phenotype for p.W25C has not been previously reported in association with ODDD, but was presented in our case.

Conclusions: Thorough examination, analysis of literature and molecular diagnosis are crucial for care improvement and further disease management, especially when patients with rare syndromes are concerned.

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P11.068D

Oliver-McFarlane syndrome: mutations of *PNPLA6* and follow-up of 30 years in two brothers

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Oliver-McFarlane syndrome (OMIM 275400) (OMFS), first described in 1965, is a rare genetic condition associating multiple and congenital pituitary hormone deficiencies (GH, TSH, gonadotropins), trichomegaly, precocious and severechorioretinal atrophy chorioretinal atrophy chorioretinal atrophy chorioretinal atrophy. If untreated, thyroid and GH abnormalities result in short stature and intellectual deficiency. Most of patients have hypogonadism, congenital or revealed during adolescence, impacting their reproduction capacity. Half of cases have progressive neurological troubles (spinocerebellar ataxia, peripheral neuropathy, spastic paraplegia). We report on 2 brothers, suspect of OMFS from childhood (Mathieu *et al*., 1991). The eldest had severe temporal and occipital alopecia, long eyelashes and eyebrows, hypolasia of tooth enamel, early chorioretinal atrophy with low vision, peripheral neuropathy, hypogonadotropic hypogonadism, GH deficiency, and short stature. The youngest had the same physical appearance, trichomegaly, less severe alopecia, enamel tooth anomalies, retinal degeneration with low vision, micropenis, testicular hypotrophy, growth retardation, GH deficiency, and osteotendinous areflexia. The 2 brothers developed spinocerebellar ataxia, with cerebellar atrophy on MRI. Two heterozygous mutations of *PNPLA6* (NM_006702) were identified [c.3241G>A / p.(Gly1081Arg) and c.3088C>T / p.(Gln1030*)]. Recent identification of biallelic mutations of *PNPLA6* gene in OMFS, confirmed the autosomal recessive inheritance suspected from many years (Hufnagel *et al.*, 2015). Biallelic *PNPLA6* mutations also induce 3 other overlapping conditions: Boucher-Neuhauser syndrome (OMIM 215470), Laurence-Moon syndrome (OMIM 245800), and Gordon-Holmes syndrome (OMIM 212840). Mutations of *PNPLA6* also caused an autosomal recessive form of spastic paraplegia (OMIM 612020). Now, the term of « *PNPLA6*-related disorders » is used to designate this group of diseases.

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P11.069A

14 years of experience of de Spanish Overgrowth Registry Consortium in the diagnostic of overgrowth disorders

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Introduction: Overgrowth syndromes (OGS) comprise a heterogeneous group in which the main feature is the generalized or partial increase of growth above 2SD. There is a high overlap of the clinical features among the OGS, making the clinical diagnosis a challenge. Since the establishment of the Spanish Overgrowth Registry (SOGRI) in 2004, more than 2,000 patients have been studied. Thus, the aim of this project is to reflect our experience in of this group of patients.

Material and Methods: This project was approved by the ethical committee of the hospital. To study the initial clinical suspicious, a battery of different methodologies were applied. For those patients who were negative for the targeted analysis, screening for other genomic alterations was performed through SNP-arrays, cGH-arrays, NGS and functional validation if necessary.

Results: The average diagnostic yield in the total syndromic and non-syndromic overgrowth was 50% and 25%, respectively. Up to date, three new entities were described: CLAPO and Tenorio syndromes and a

ch19p13.3 microdeletion/microduplication syndrome. Also, our group was involved in the development of several clinical guidelines for overgrowth syndromes.

Conclusions: Overall, the molecular confirmation of an initial clinical suspicious was about 50% in the SOGRI cohort. Three new clinical entities and their underlying molecular defects were described, highlighting the importance of the application of new technology to study the negatives cases. There are a relatively high number of patients without molecular confirmation, suggesting the existence of new molecular mechanism that remain unknown and must be explore in the future.

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P11.070B

Autosomal dominant recurrent parotitis: an under-reported entity

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Familial salivary glands inflammation is a rare condition of unknown aetiology, affecting mostly parotid glands. Only a single British pedigree is referenced in OMIM. By evaluating a 34 y-old French female patient suffering chronic recurrent parotitis since the age of 6 years, we uncovered a similar involvement in her father and her son, making autosomal dominant (AD) inheritance likely. In addition the patient had a progressive distal four limbs muscle wasting, paresis and camptodactyly corresponding on nerve biopsy to focal alterations of the myelin sheet suggestive of a demyelinating peripheral neuropathy, contrasting with apparently normal NCV. We are unable to conclude if the motor impairment is coincidental or belonging to the clinical spectrum of AD chronic parotitis since her affected relatives had no motor impairment. Another family evaluated in Belgium was also suggestive of AD inheritance. A careful search in medical literature identified seven similar additional families, all of them being compatible with an AD mode of inheritance. Unfortunately, the corresponding papers were too old to allow a reevaluation. Based on WES and segregation analysis performed in the current two pedigrees, a variant in an interesting candidate gene was identified. Additional families will be necessary to confirm this preliminary result.

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P11.071C

A new recognizable syndrome caused by mutations in the *PITX1* gene

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We report the clinical and molecular characterization of a previously undescribed syndrome observed in a family (mother and son) and an unrelated child.

Patient 1, aged 3.5 years, presented with mandibular hypoplasia, knee flexion deformity, hyperlordosis, and prenatal-onset short stature (-2,5SD). Psychomotor development was normal as were cardiac and renal ultrasounds scans, and chromosomal microarray. His mother (patient 2) showed a similar phenotype: mandibular hypoplasia, knee instability requiring surgery, hyperlordosis, short stature treated by growth hormone (adult height: 160 cm), recurrent otitis during childhood, and myopia. Patient 3 was an unrelated child presenting with Pierre-Robin sequence and patellar agenesis leading to knee flexion deformity. Psychomotor development and cardiac/renal ultrasounds

scans were normal. Radiological features included a striking mandibular obtuse angle and narrow iliac wings.

Whole exome sequencing detected heterozygous missense mutations in the homeobox transcription factor *PITX1* gene (c.793G>T in patients 1 and 2; c.412A>C in patient 3 and, in a mosaic status, in his asymptomatic father). *PITX1* anomalies have been reported to cause different syndromes: i) Liebenberg syndrome, characterized by malformations of upper limbs that acquire radiological features of the legs, caused by 5q31.1 rearrangements involving putative *PITX1* regulatory elements by disruption of a TAD; ii) Mirror-image polydactyly/tibial agenesis, caused by intragenic deletion; iii) Congenital clubfoot with or without preaxial polydactyly and right tibial hemimelia, caused by dominant negative mutation. The patients here described showed a distinct recognizable allelic picture, whose hallmarks are mandibular hypoplasia, narrow iliac wings, patellar hypoaplasia leading to knee flexion deformity, and possible short stature.

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P11.072D

Poland Sequence - long time follow-up of 21 cases

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Poland sequence is a rare disorder that associates unilateral defect of pectoralis muscle and syndactyly of hand on the same side. The disorder is considered "a non-specific developmental field defect" occurring at 6 weeks of fetal development, for the moment the cause being unknown. It is suggested that diminished blood flow through the subclavian artery that goes to the arm may be the precipitating cause. Rare cases are thought to be caused by a genetic change that can be passed down in families, but no related genes have been identified. We have performed a clinical study on 21 cases of Poland sequence diagnosed in Iasi Medical Genetics Center, aiming to identify defects associated to the main features, as well as the clinical evolution. Our group included 13 males and 8 females. Thirteen patients had right-sided Poland's syndrome, eight left-sided. Two patients were related (brothers). Major clinical findings include: hypoplasia/aplasia of pectoralis muscle 21/21 cases, symbrachydactyly 12/21, hypoplastic/absent nipple 5/21, hand hypoplasia 4/21, scoliosis 3/21 and forearm hypoplasia and rib defect 1/21. Occasional findings include: congenital heart defect found in 7/21 cases, spasmophylia 2/21 and cleft lip/palate in 1/21 cases. Genetic tests have been normal. The evolution in time has been constant. In conclusion, Poland anomaly is more common in boys than girls, and the right side is affected approximately twice as often as the left. Most cases arise sporadically. However, because we have identified an affected sibship, we appreciate that in-depth genetic testing should be performed.

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P11.073A

Redefining the mutational spectrum and gene-phenotype correlates in pontocerebellar hypoplasia: results of a multicentric Italian study

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Introduction: Pontocerebellar hypoplasias (PCH) comprise a heterogeneous group of inherited autosomal recessive or X-linked disorders characterized by concurrent hypoplasia of the pons and the cerebellum and variable clinical and imaging features. The current classification includes 11 PCH subtypes, and 20 associated genes are known to date.

Materials and methods: We performed deep clinical and imaging phenotyping in 62 Italian probands (39 females) with neuroradiological diagnosis of PCH, who underwent NGS-based panel sequencing of all known PCH-associated genes (TruSeq Custom Amplicon technology on a MiSeq platform) and MLPA for CASK exon rearrangements.

Results: The responsible genetic defect was identified in 44 probands (71%). Interestingly, the commonest causative gene was *CASK* (40%), which harbored both SNVs and CNVs and was mutated in females and males, with striking genotype-phenotype correlates. The European founder

mutation *TSEN54* p.A53T and pathogenic variants in *EXOSC3* only accounted for 18% and 8% cases, respectively. Single patients with peculiar phenotypes were mutated in *RARS2*, *VLDLR* and *TOE1*. We confirmed some previously reported associations, e.g. retinopathy and microcephaly with *CASK*, lower motor-neuron signs with *EXOSC3* and cerebellar cysts with *TSEN54*. However, we could not replicate other gene-phenotype correlates: for instance, we failed to observe univocal correlations of *TSEN54* p.A53T with hyperkinetic movement disorders, nor with the typical "dragonfly appearance" of cerebellar hemispheres.

Conclusions: *CASK* represents the major gene causative of PCH in Italy. Phenotypic variability of PCH subtypes is wider than previously thought, with significant clinical and neuroimaging overlap among distinct conditions.

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P11.074B

Assessment of genome-wide burden of rare genic CNVs in posterior fossa malformations

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Introduction: Posterior fossa malformations (PFMs) include a wide spectrum of congenital abnormalities, heterogeneous with respect to neuroimaging findings, genetic cause and recurrence risk. The contribution of genomic Copy Number Variants (CNVs) has been poorly investigated so far, making it difficult to counsel families as regard this genetic test.

Materials and methods: CNVs were assessed either by CGH- or SNP-array in a cohort of 111 probands with various PFMs. Detailed neuroimaging assessment led to classify the patients in the following groups: Dandy-Walker malformation (DMW, $n = 10$), isolated vermis hypoplasia (IVH, $n = 12$), whole cerebellar hypoplasia (WCH, $n = 12$), cerebellar dysplasia (CD, $n = 18$), ponto-cerebellar hypoplasia (PCH, $n = 18$), non-progressive cerebellar atrophy (NPCA, $n = 13$) and other PMFs ($n = 28$). A logistic regression model was used to assess the proportion of CNVs in PFM classes.

Results: Pathogenic heterozygous CNVs (all deletions) were identified in 9 (8%) probands, including 5 (50%) DWM, 1 (8%) IVH, 1 (8%) WCH and 2 (11%) CD. No CNVs were detected in the PCH and NPCA groups. CNVs were significantly commoner in DWM than in non-DWM (log-odds = 3.078, $p < 0.001$), while their frequency was not significantly enriched in the other PFM categories. The identified genomic deletions were in most cases associated to a syndromic phenotype. Deletion of 3q24 region was confirmed as a DWM-associated CNV.

Conclusions: Screening is being extended to a larger cohort of PFMs. CNVs analysis is advised in patients with DWM, and in other PFMs when associated to a syndromic phenotype.

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P11.075C

Co-occurrence of two recurrent copy number alterations in a child with complex phenotype

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Introduction: two copy number variations observed on the same chromosome in a patient is a very rare condition. In our patient beside of the 17p11.2 microduplication an additional deletion of 17q12 region has been detected.

Methods: routine cytogenetic investigation was indicated in a 8 months old baby because of neonatal hypotonia, developmental delay, renal cysts, hearing loss and minor

anomalies which resulted in normal karyotype. Because of the complex clinical symptoms array CGH analysis using Agilent Sureprint 8x60K oligo-array (ISCA v.2) was performed.

Results: array CGH detected a 3,573 Mb recurrent microduplication of 17p11.2 and additionally a 1,638 Mb deletion affecting 17q12. The unexpected results provided explanation of the complex clinical features allowing for a more detailed genotype-phenotype analysis. Our patient has been diagnosed as having Potocki-Lupski syndrome and Renal cysts and diabetes syndrome at the same time.

Conclusions: the high-resolution array CGH analysis in patients with a complex phenotype is of great importance and it is recommended to carry-out this test first. The application of array CGH in patients with unusual compound phenotype may enable more accurate estimates of the incidence of similar cases and can lead to the exploration of development of multiple copy number alterations in the same patient.

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P11.076D

Primrose syndrome: a phenotypic comparison of patients with a *ZBTB20* single-nucleotide variant versus a 3q13.31 microdeletion including *ZBTB20*

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Primrose syndrome is characterized by variable intellectual deficiency, behavior disorders and facial dysmorphism with macrocephaly. The phenotype is progressive with distal muscle wasting, contractures, hearing loss and ectopic calcifications of the ears and brain. In 2014, *ZBTB20 variants* were identified as responsible of Primrose syndrome. Indeed, *ZBTB20* plays an important role in cognition, memory, and learning processes, and has a transcription repressive effect on numerous genes. A more severe, progressive phenotype was found in a small number of patients with single nucleotide variants (SNVs) than in those with large deletions. Here, we report on the clinical and molecular results of 14 patients, seven carrying *ZBTB20* SNV, and seven carrying 3q13.31 deletions, recruited through the French AnDDI-Rares network. We compared their phenotypes and reviewed data in the literature, in order to establish more powerful phenotype-genotype correlations between the two groups of patients. All patients presented mild-to-severe ID and/or a psychomotor delay. Facial features were similar with a prominent forehead, down-slanting palpebral fissures, ptosis and large ears but macrocephaly was more frequent in patient with SNVs ($p = 0.026$). Hearing loss ($p = 0.004$), pinna calcification and progressive muscular wasting and contractures were observed only in patients with SNVs. Corpus callosum dysgenesis ($p = 0.003$), diabetes and hypothyroidism were more frequent in this group. However, the median age was 9.7 years in patients with deletions compared with 17.5 years in those with SNVs. Longer follow-up will be necessary to determine whether the phenotype of patients with deletions is also progressive, and to adapt information given to families.

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P11.077A

PTK7-a candidate gene for a novel human malformation phenotype

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Introduction: Prenatal ultrasound identifies an increasing number of fetal malformations. We report on a fetus with brain malformations, Müllerian duct aplasia, bile duct atresia, unilateral anophthalmia and bilateral cleft palate. Our aim was to identify the genetic cause for this novel multiple congenital anomaly (MCA) phenotype.

Methods: We perform trio whole exome sequencing (WES) and prioritize variants according to their frequency, disruptive potential and their presence in genes involved in early developmental processes. We correlate the malformation pattern, confirmed by autopsy, to the candidate genotypes. The additional comparison of human and animal morphology validates potential candidate genes. Using RT-PCR and qPCR we study expression on fetal FFPE-RNA. We are further investigating the specific mutations in a cell culture expression model.

Results: We identified candidate missense mutations in the *PTK7* gene which encodes a membrane receptor tyrosine kinase acting in the canonical and non-canonical Wnt-signaling as well as the planar cell polarity pathways. So far, the gene is known to play a role in Müllerian duct and neural tube development. Knockout mice embryos present with a similar severe malformation pattern. qPCR of liver tissue of the fetus confirmed a decrease of PTK7 mRNA.

Conclusions: *PTK7* is a candidate gene for a novel human MCA syndrome. WES can be used in individual families with undiagnosed lethal MCA syndromes to discover novel disease genes, provided that the fetal phenotype can be correlated to a particular developmental pathway in embryogenesis. Proof of pathogenicity of the mutations in such ultrarare disorders can be challenging.

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P11.078B

Pura syndrome: an emerging neurodevelopmental disorder

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PURA Syndrome is a neurodevelopmental disorder characterized by moderate/severe cognitive impairment, delayed or absent speech and difficulties in acquiring independent ambulation. At birth, newborns present central hypotonia, hypothermia, lethargy, swelling-feeding difficulties,

frequent hiccups and respiratory abnormalities that may need intensive care for life-threatening risk. In the 90% of the patients a heterozygous mutation in PURA gene is present, revealed mostly through WES analysis; in the remaining 10% a deletion of 5p31.3 encompassing the PURA gene is detected using Array-CGH. Until last year, 71 patients had been described, but the number is rapidly growing. We report 4 Italian patients (age range 6 month - 11 years). At birth, all of them exhibited axial hypotonia, swallowing difficulties and hypoventilation. Two had neonatal respiratory failure requiring intensive care and recurrent hiccups. Recurrent dysmorphic features were anteverted nostrils, thin and sparse eyebrows. Neurodevelopment was delayed in all patients, with absent speech and limited walking autonomy in older patients. De novo heterozygous mutations in PURA gene were found in 2/4 patients by WES, using an NGS panel in 1 patient and a de novo 2,6 Mb deletion in 5q31.2q31.3 was detected using array-CGH in the remaining patient. PURA Syndrome, is now a frequently recognized neurodevelopmental disorder which should be considered in the differential diagnosis of syndromes manifesting with the neonatal hypotonia and early respiratory abnormalities.

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P11.079C

Analyzing 66 cases of RASopathies with an extended NGS panel

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Introduction: RASopathies are a group of genetic diseases caused by mutations in genes encoding proteins in the Ras/MAPK pathway. RASopathies include several syndromes: neurofibromatosis-1, Noonan/LEOPARD, Costello, Legius and cardio-facio-cutaneous syndromes, etc. The phenotypic spectrum is wide and overlapping.

Material and Methods: We present a cohort of 66 patients from different populations with suspicion of RASopathies. We used an extended NGS panel (TruSight-One, Illumina) including 13 RASopathies associated genes. Other 32 candidate genes were selected by R custom script on PSIQUIQ library, using molecular interaction databases (Biogrid, IntAct, MINT, Reactome, UniProt, etc.) regarding to previous 13 genes.

Results: We detected pathogenic/probably pathogenic variants (P/PP) in the 35%(23/66) of the patients, in the 41% (27/66) were detected one or several variant of unknown significance (VUS), and in the 24% (16/66) no variants of interest (VI) were identified. 88 VI (P/PP or VUS) were detected in 26 different genes, *PTPN11* was the most mutated gene (15% of variants, 12 P/P and 1 VSI) followed by *ANKRD11* (10%, 9 VSI), *SRCAP* (9%, 8 VSI) and *SOS1* (8%, 3 P/PP and 4 VSI). The 65% of VI (all classified as VUS) was detected in candidate genes.

Conclusions: We show the results of applying an extended NGS panel in a cohort of 66 patients. According to the literature, *PTPN11* and *SOS1* were the most mutated RASopathies related genes. Remarkably, we detected a high percentage of VUS in candidate genes that could have clinical implications. However, further familiar and functional analysis must be done to confirm this.

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P11.080D

The smallest SATB2 exon-deletion detected by arrayCGH. Further delineation of a new emerging syndrome

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Introduction: Few cases of recurrent 2q33.1 deletion with size variability have been reported in the literature. Haploinsufficiency of one gene, SATB2, within the deleted region has been suggested to be responsible for most of the features of the affected patients and a new syndrome named SATB2-associated syndrome (SAS) has been proposed. Herein, we present a case with the smallest SABT2 deletion detected by arrayCGH, to our knowledge.

Patient and Results: The present case is a boy evaluated in the Pediatric Neurology consultation at the age of 3 years with developmental growth retardation. Conventional chromosome analysis of the patient revealed a normal karyotype. X-fragile was normal and an array CGH analysis was performed. The CytoSure™ Constitutional v3 4x180k (Oxford Gene Technology, UK) was used, scanned using the Agilent Microarray Scanner according to the manufacturer's protocol. High-resolution microarray analysis of the patient detected a 5,24 Kb deletion in the region 2q33.1, arr

[GRCh37]2q33.1(200212030_200217267x1) including exon 8 of SATB2 gene. The main features of the patient were concordant with those related to SAS: intellectual deficit, autistic traits, hypotonia, elongated face, atypical teeth, micrognathia, local hypertonia, atypical thumbs and nails, sparse hair.

Comments:

- Intragenic SATB2 deletions are very rare and only four cases have been described. Review of clinical records showed similar clinical features among these patients, including severe developmental delay and tooth abnormalities as the present one.

- CytoSure™ Constitutional v3 array offers an enhanced exon-level coverage and has allowed the detection of the smallest SATB2 deletion described until now.

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P11.081A

Identification of new biomarkers for *MAGEL2* truncating mutations in Schaaf-Yang syndrome

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Schaaf-Yang syndrome (SHFYNG) is due to truncating mutations in *MAGEL2*, a gene included in the Prader-Willi region (15q11-q13). This syndrome is characterized mainly by neurodevelopmental delay, contractures and craniofacial dysmorphology and around 100 patients have been identified worldwide so far. MAGEL2 is an essential component of the retromer, involved in the retrograde transport (back to the trans-Golgi network). Previous studies have shown that the depletion of MAGEL2 leads to a change in the subcellular localization of integrin alpha-5 and that the alteration of VPS35, a MAGEL2 partner, leads to alterations in the APP transport in the endosome. We have analysed different biomarkers in fibroblasts of 3 SHFYNG patients in comparison with 6 healthy controls. Intracellular localization of Integrin alpha-5 was assessed by immunocytochemistry while the levels of the amyloid-beta (1-40) were measured by ELISA. Cell viability was measured by the MTT assay. We observed no difference in the viability of the fibroblasts of the patients when compared with controls and no alteration in the integrin alpha-5 levels in the cellular membrane while we notice a slight increase in integrin alpha-5 co-localization with early endosomes (marked with EEA1). We have observed a significant decrease of the AB_{1-40} in the patient's fibroblasts when compared with controls. In conclusion, we have identified a promising biomarker (AB_{1-40}) that could help to better understand the pathophysiology of the SHFYNG syndrome and to monitor the effect of therapeutic drugs. Grants: Spanish Ministerio de Economía y Competitividad (SAF2014-56562-R; FECYT, crowdfunding PRECIPITA). Catalan Government (2014SGR932)

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P11.082B

CASE REPORT OF SHAAF-YANG SYNDROME WITH A DE NOVO MUTATION IN MAGEL2 GEN

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Introduction: Shaaf-Yang syndrome (SHFYNGS; #MIM 615547) is an autosomal dominant multisystem disorder characterized by delayed psychomotor development, intellectual disability, hypotonia, and behavioral abnormalities. Additional features include contractures, feeding difficulties, and variable dysmorphic facial features. MAGEL2 is located in the Prader-Willi critical region 15q11-13, and have been reported to cause SHFYNGS. Individuals are affected only if the mutation occurs on the paternal allele, since MAGEL2 is a maternally imprinted gene.

Case Report: Female patient with polymalformative syndrome, fetal hypokinesia, arthrogryposis, small feet, hypotonia, and psychomotor retardation. At[AZC1] 22 months of age, she was not able to keep standing without support, had not speech development, clamp nor steps. She developed trunk obesity, not associated with increased intake. She did not show hyperphagia and presented feeding problems. No alterations in brain, spinal, abdominal, renal or cardiac MR imaging studies. Normal array-CGH. Metabolic study including HBA1c, lipid metabolism, liver function and cortisol normal. Genetic study was performed by PCR amplification of exonic and intronic regions of MAGEL2 gen, and analyzed by Sanger sequencing method. An heterozygous variant was detected, c.1850G>A (p.Thr617Ter; NM_01966) and it was de novo since parents were not carriers. Reported cases of SCHYNGS inherited usually a paternal mutated allele. In the absence of paternal deletion of 15q11-15q13 or maternal uniparental disomy 15, a search for intragenic mutations on the paternal allele of MAGEL2 should be proposed for fetuses with reduced movements, polyhydramnios, and

distal arthrogryposis, newborns with severe undiagnosed central hypotonia, or children for whom PWS is clinically suspected. [AZC1]

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P11.083C

Utilizing facial recognition software in individuals with Sifrim Hitz Weiss Syndrome

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Introduction: Sifrim-Hitz-Weiss syndrome (SIHIWES) is a recently described form of syndromic intellectual disability associated with congenital heart defects, hypogonadism, macrocephaly and additional features. The condition is caused by *de novo* missense variants in *CHD4*, which encodes an ATP-dependent chromatin remodeler. Most reported variants are missense and lie within the ATPase/helicase domains. A few variants were identified outside of this hotspot region and therefore their effect is unclear. The aim of this study was to investigate whether facial recognition software can be utilized for facial gestalt recognition and aid in variant interpretation in SIHIWES.

Methods: We used the deep convolutional neural network architecture provided by Face2Gene (FDNA Inc, USA) on 16 photographs of individuals with variants in the ATPase/helicase domain of CHD4. In addition, a comparison of SHINWES individuals to control groups was conducted using across-validation approach. Then, we evaluated the ability of the software to recognize the SIHIWES gestalt in two individuals with *de novo* variants outside of the hotspot region.

Results: The cohort of individuals with SIHIWES and variants in the ATPase/helicase domain differed significantly from a control group of 32 healthy individuals (AUC 0.917, p-value 0.009). For the two individuals with variants outside of the ATPase/helicase domains, SIHIWES was the second and fifth top syndrome suggested by the software supporting these variants result in a similar phenotype.

Conclusions: Facial gestalt recognition is sometimes used by clinicians in the interpretation of novel variants in disease causing genes. Here we show this can also be applied by facial recognition software.

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First Prenatal Diagnosis of a Rare STAR Syndrome

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Introduction: STAR syndrome (Syndactyly, Telecanthus, Anogenital malformations, Renal malformations) is a very rare x-linked dominant disorder. Loss-of-function mutations of the FAM58A gene were previously reported to be associated with STAR syndrome. Less than 20 patients have been described thus far, two of them displaying mild developmental delay.

Case presentation: The parents were referred to genetic counseling at 27 weeks of gestation during their first pregnancy due to a sonographic demonstration of absent gallbladder. No consanguinity or abnormal medical conditions were reported, and the pregnancy was uneventful. As part of genetic evaluation, the parents were tested for Cystic Fibrosis, and fetal Chromosomal Microarray Analysis (CMA) was recommended.

Results: CMA testing yielded a female karyotype with Xq28 deletion sized 117kb, which included 3 clinically significant genes - FAM58A gene (raising a suspicion of STAR syndrome) and two genes related to recessive disorders. Parental CMA testing was normal. Magnetic Resonance Imaging was performed, yielding no abnormal findings. Following a throughout discussion, the parents decided to continue the pregnancy. The newborn girl was delivered at 38 weeks of gestation. She was diagnosed with unilateral 2-4 toe syndactyly, with no evidence of dysmorphism or renal/anogenital malformations. At the age of 2 months the parents report normal congenital and motor development.

Conclusions: To the best of our knowledge, we describe the first prenatal diagnosis of STAR syndrome, with mild abnormal features. This case is an interesting example of a challenging genetic counseling in the face of very limited clinical information.

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P11.085A

Stormorken syndrome and York platelet syndrome share the same mutation of *STIM1* and the same ultrastructural platelet anomalies

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In 1985, Stormorken *et al.* reported a unique family with a new phenotype associating thrombocytopathia, thrombocytopenia, muscle fatigue, asplenia, miosis, migraine, dyslexia and ichthyosis. In 2000, Mizobuchi *et al.* found tubular aggregates on the muscle biopsy in another family with this phenotype. In 2014, three groups including ours, found that « Stormorken syndrome » (OMIM 185070) was related to a specific gain-of-function mutation in *STIM1* gene [c.910C>T/p.(Arg304Trp)]. *STIM1* encodes a calcium sensor involved in Store-operated calcium entry. With the mutation, this system seems permanently activated, even when intracellular stocks of calcium are full. Independently, White *et al.* reported from 2003 a new platelet disease, characterized in electronic microscopy by the presence of 2 types of platelet organelles (opaque and target organelles). In 2015, they found that "York platelet syndrome" was also caused by mutations of *STIM1* gene, including the c.910C>T [p.(Arg304Trp)] (Markello *et al.*, 2015). The patients shared several symptoms with patients affected by Stormorken syndrome: thrombocytopathia, thrombocytopenia, muscle weakness, rimmed vacuoles on muscle biopsy, and sometimes miosis or splenic hypoplasia. As the 2 conditions were secondary to the same mutation of *STIM1* gene, and patients had a rather similar phenotype, we assumed that they constituted one unique disease, only investigated by different approaches. In this work, we showed for the first time that ultrastructural platelet anomalies described in York platelet syndrome, were also found in platelets of patients with Stormorken syndrome. This result suggests that York platelet syndrome and Stormorken syndrome are one and the same disease.

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P11.086B

Detection of mosaicism involving structural and numerical chromosome aberrations from targeted sequencing data

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Mosaicism is defined as the presence of two or more genetically distinct cell lines. With the introduction of increasingly sensitive technologies for DNA mutation detection such as microarrays and next-generation sequencing, the importance of mosaicism for human diseases is now being more fully appreciated. However, extracting information on mosaicism from targeted sequencing, especially exome data, can be challenging because read coverage varies between regions and the target is limited to ~ 3% of the genome. In the present study, we aimed to evaluate the efficiency of a new targeted approach to detect mosaicism involving structural/numerical chromosomal alterations of DNA samples previously characterized by SNP-array. We used a focused exome panel supplemented by backbone and SNPs probes that express a larger representation of the human genome. A total of 9 DNA mutations ranging from 400 Kb to 35 Mb and with different level of mosaicism (10-50%) were used as positive control for targeted sequencing analysis. Our approach correctly identified 7 alterations (7/9), pointing to a sensitivity of 81% of our test. The two cases that were not detected are below 30% of mosaicism. Although in both cases it was possible to observe a greater dispersion of probes that deviated from the 50% heterozygosis line, the algorithms did not correctly integrate the information of copy number and B allele frequency to make a call. Our results point out that it is possible to improve the methodology for the identification of structural mosaicism from sequencing data based on the analysis of allelic frequency.

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P11.087C

Sweeney-Cox syndrome: report of the third patient affected by this new delineated syndrome harboring a novel variant in TWIST1

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Introduction: Sweeney-Cox syndrome (SCoS) is a new syndrome reported in two individuals presenting heterozygous missense variants in residue 117 in *TWSIT1*, showing a dominant-negative effect. Previously, heterozygous mutations in this gene, leading to haploinsufficiency, were known to cause Saethre-Chotzen syndrome (SCS). In SCoS, the craniofacial involvement is

fundamentally different from the one seen in SCS and premature closure of cranial sutures has not been a hallmark.

Casuistic and Methods: the proband is a 4 year-old male patient showing typical facial features of SCoS and coronal craniosynostosis. A targeted gene panel for craniosynostosis was performed in the proband, followed by Sanger sequencing in the proband and his parents.

Results: a novel, *de novo*, missense variant (p. Asp141Glu) in *TWIST1* was found in the proband.

Conclusions: This is the third patient reported with SCoS. Although the facial features are typical for this new recognized syndrome, the patient presented with coronal craniosynostosis, similar to what is seen in SCS, suggesting that this specific variant could cause a phenotype comprising clinical characteristics of both SCoS and SCS. The variant found in *TWIST1* changes the aminoacid aspartate for a glutamin in position 141. Different variants in the same residue have been reported associated with SCS. It is possible to speculate that a substitution for a more similar aminoacid, as it is the case here, could lead to a dominant-negative effect, similar to what was proposed for the variants in residue 117. Further functional studies are required to prove this preliminary impression. FAPESP 2015/21783-9; 13/08028-1; CNPq 304130/2016-8

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P11.088D

TARP syndrome: clinical characteristics of a 10 year-surviving patient and review of the literature

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Introduction: TARP syndrome, comprising Talipes equinovarus, atrial septal defect, Robin sequence, and persistence of the left superior vena cava, is a severe X-linked condition that has been considered lethal in affected males. The disease is caused by inactivating mutations in *RBM10*, which encodes for a RNA binding motif protein involved in regulation of alternative splicing of diverse mRNA precursors.

Materials, Methods and Results: By using a trio-based WES approach, coupled with an in-house implemented pipeline, we identified a maternally inherited *RBM10* frameshift variant in a survived 10-year-old male with clinical features fitting TARP syndrome. The *RBM10* variant was confirmed by Sanger and was documented to cause aberrant processing of the transcript with complete loss of the *RBM10* mRNA.

Conclusions: To our knowledge, this is the second individual with TARP syndrome who survived at the first decade of life, allowing a first depiction of the natural history of this disorder. These data indicate that an adequate intensive neonatal care can overcome the supposed lethality of TARP. This should to be taken into consideration in counselling.

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P11.089A

Whole Exome Sequencing reveals novel biallelic *UBE3B* mutations in two unrelated patients with undiagnosed condition

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We report two unrelated patients with severe congenital malformations and distinctive facial appearance, for whom we have primarily hypothesized Marden-Walker syndrome in the first case and CHARGE syndrome in the second one. In both cases, exome sequencing (WES) was performed in order to validate our clinical suspicion. WES revealed novel biallelic variants in the *UBE3B* gene: in the first case we found homozygosity for a novel truncating mutation (c.2169_2170insG p.Ile725Aspfs) within the HECT domain of UBE3B protein, while in the second case compound heterozygosity for two novel missense mutations (c.214A>G p.Ser72Gly; c.838T>C p.Ser280Pro). Biallelic mutations of *UBE3B* have recently been associated with Kaufman oculocerebrofacial syndrome (KOS), a rare entity within the blepharophimosis-metal retardation syndromes, characterized by typical clinical features but often misdiagnosed. Affected subjects present microcephaly, poor growth, respiratory difficulties, gastrointestinal and genitourinary anomalies and a peculiar constellation of facial

features, like blepharophimosis, hypertelorism, depressed nasal bridge, low-set and posteriorly rotated ears and micrognathia. To date only 19 patients from 16 unrelated families were reported and in all cases an initial different diagnosis were made. KOS represents a rare disease that, despite showing a characteristic phenotype, represents a challenging clinical diagnosis based only on clinical aspects. WES allowed us to make a diagnosis of KOS in our cases reducing cost and time of the diagnostic pathway, demonstrating, once again, its capability in establishing the clinical diagnostic rate, and its impact on medical management in a large paediatric centre. WES, moreover, provides a unique glimpse into the complexity of genetic disorders.

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P11.090B
Overlapping phenotypes in patients harboring heterozygous mutations in KAT6A and KAT6B

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Introduction: *KAT6A* and *KAT6B* encode a lysine acetyltransferase, playing a role in chromatin regulation. Heterozygous variants in *KATB* have been associated with Say-Barber-Biesecker-Young-Simpson and genitopatellar syndromes (GPS), both showing developmental delay/mental retardation and dysmorphic features. Although these two disorders were well characterized syndromes, some patients present overlapping clinical features, hampering a straightforward genotype-phenotype correlation. Heterozygous variants in *KAT6A* have been recently associated with mental retardation, microcephaly and variable dysmorphic features. Patellar anomaly is considered a cardinal feature of GPS within this group.

Casuistic and Methods: we report on three non-related individuals, two female patients with typical findings of GPS, one of them with preaxial polydactyly, and no family history for polydactyly, and a male patient with severe developmental delay, absent speech, microcephaly, facial dysmorphisms and patellar hypoplasia. A targeted gene panel and sanger sequencing was performed in the two female probands and whole-exome sequencing in the male proband.

Results: frameshift variants in *KAT6B*, (p.S1303Vfs*31) and (p.K1199Rfs*26), were found in the female patients. A nonsense variant in *KAT6A* (p.R1129*) was found in the male patient. Sanger sequencing confirmed a *de novo* event in the latter.

Conclusions: this is the first patient with *KAT6A* mutation showing patellar hypoplasia. Due to the similarity of function between *KAT6A* and *KAT6B*, this result could expand the phenotype of this autosomal dominant mental retardation syndrome, suggesting the disorder has overlapping features with GPS. Moreover, one of the proband with GPS presented with preaxial polydactyly, which has not been previously reported. FAPESP 2015/21783-9; CNPq 304130/2016-8

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P11.091C
Dual overlapping phenotype recessively inherited due to paternal unipaternal disomy of chromosome 2 (pUPD2) in a patient

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Introduction: Blending of two different disease phenotypes in a single patient may apparently suggest a new clinical phenotype or prevent the diagnosis of the other. Whole-exome sequencing (WES) can provide insight into the relationship between observed clinical phenotypes and underlying genotypes. Here we present, a patient with both Warburg Micro syndrome-1 (WARBM1) and Hypotonia, infantile, with psychomotor retardation and characteristic facies 2 syndrome (IHPFR2) caused by pUPD2 detected by WES analysis.

Patient and Results: WES analysis of a 14-year-old male patient whose parents are unrelated, with characteristic WARMB findings revealed two novel homozygous mutations in both of the RAB3GAP1 (c.664delC) and UNC80 genes (c.C1459A). Sanger sequencing confirmed that the same mutations was present in the patient's heterozygous father but not in the patient's mother. In the homozygosity analysis, it was found that pUPD2. In the patient, because of overlapping findings of the both syndrome, the presence of IHPFR2 syndrome in the patient was undiagnosed until the end of the analysis.

Conclusion: To date, the UPD2, especially of paternal origin, has been very rarely described in the literature. When considering literature data there are no paternally

imprinted genes on chromosome 2 that have a major effect on growth or development. With the exception of various autosomal recessive disorders their phenotype is normal. In patient with multiple molecular diagnoses, the phenotypic complexity of disorder can make it difficult for doctors to diagnose it. Genomewide analyses may reveal more than one Mendelian disease that is relevant for a patient and the patient's family.

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P11.092D

Towards a novel diagnostic strategy using patient-derived URECs to diagnose ciliopathies

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Introduction: Diagnostic exome analysis frequently leads to an inconclusive molecular diagnosis due to the detection of variants of unknown significance. Functional tests evaluating the effect of genetic variants on protein level could elucidate their pathogenicity. Urine-derived renal epithelial cells (URECs) provide a non-invasive source of patient material. We used patient-derived URECs to functionally evaluate the pathogenicity of genetic variants potentially associated with ciliopathies (disorders caused by cilium dysfunction).

Materials and methods: URECs were obtained from patient's urine and used for functional tests: (1) the ciliary phenotype was analyzed using immunofluorescence cytochemistry and (2) URECs and blood cells were used to study mRNA splicing.

Results: We tested the pathogenicity of two heterozygous variants of unknown significance in *IFT140*, identified by WES in a Mainzer-Saldino syndrome patient. *IFT140* encodes a protein involved in ciliary intraflagellar transport (IFT). Patient-derived URECs showed abnormal IFT in comparison to healthy controls, which confirmed the pathogenicity of both variants and validated the patient's diagnosis. We also studied mRNA splicing of *DYNC2H1* in another ciliopathy patient with a heterozygous synonymous change. *DYNC2H1* encodes a subunit of the IFT dynein motor. mRNA splicing revealed exon skipping and premature transcription termination. Together with the pathogenic variant on the second allele, this functional assay helped to diagnose this patient.

Conclusion: The increasing number of variants with uncertain pathogenicity requires functional testing to improve diagnostics. Functional tests using urine-derived patient cells have proven to be an attractive non-invasive procedure to facilitate accurate diagnosis of ciliopathy patients.

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P11.093A

***SHROOM4* as a candidate gene for VATER/VACTERL association and characterization in a zebrafish model**

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The acronym VATER/VACTERL association refers to the rare, non-random co-occurrence of the following component features (CFs): vertebral defects (V), anorectal malformations (ARM) (A), cardiac defects (C), tracheoesophageal fistula with or without esophageal atresia (TE), renal malformations (R), and limb defects (L). Patients may present with additional congenital anomalies however, the clinical diagnosis requires the presence of at least three CFs. The pathogenesis of VATER/VACTERL association is heterogeneous and remains to further be elucidated.

In a multiplex family with VATER/VACTERL association, we performed whole exome sequencing (WES). Pedigree information suggested an underlying X-chromosomal recessive mode of inheritance which appeared likely since the index female patient showed skewed X-inactivation. We detected her maternally-derived X chromosome being activated in 84% of lymphocytes, while array-based analyses were normal. Consecutive WES and filtering for rare X-chromosomal variants prioritized one novel variant in *SHROOM4* (p.E314K).

We are currently re-sequencing *SHROOM4* in 310 male patients with VATER/VACTERL, VATER/VACTERL-like, A only, or TE only phenotype utilizing a targeted re-sequencing approach with molecular inversion probes. Expression studies of *shroom4* in zebrafish larvae (zfl) showed expression from 48 until 72 hours post fertilisation in the region of the terminal gut. For functional analysis, we have initiated Morpholino-knock-down (MO) experiments in zfl to describe morphological changes in Shroom4 MO-morphants. Preliminary data suggests increased death rates and a clear morphologic alteration of the cloaca, as well as differences in fluorescent in vivo dye uptake and excretion

assay. However, the findings are not yet fully conclusive and further analysis is warranted.

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P11.094B

Urinary tract abnormalities associated with Waardenburg Syndrome due to mutations in EDN3 gene: a new phenotype

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We report the case of a male newborn come to our observation for the evidence, at birth, of a white forelock of hair.

Family history reported several cases of unspecific premature graying of hair (onset in childhood-adolescence). The parents were first degree cousins. During pregnancy, bilateral hydroureteronephrosis, megacystis and ureteral stenosis were observed at the XXth ww prenatal ultrasounds examination. Karyotype and Array CGH on amniotic fluid were normal. In the first days of life, abdominal distension became evident and failure of meconium passage was observed. He underwent several multiple surgery interventions due to subsequent intestinal blocks. Intestinal biopsy was suggestive for Hirschprung disease. Ophtalmologic evaluation was normal. Otoemissions revealed bilateral profound sensorineural hearing loss. Renal and urinary tract instrumental investigations confirmed the prenatal findings. He underwent a surgical intervention of right ureteral reimplantation. At 50 days he developed epileptic seizures. Central hypotonia and psychomotor delay were observed.

In the strong suspicious of a Waardenburg Syndrome (WS) type 4, molecular analysis of EDN3, EDNRB and SOX10 genes was performed. This analysis revealed an homozygous nonsense variant c.364G>T (p.Glu122*) in EDN3 gene, likely pathogenic but never described in literature, inherited from both his parents.

This is the first case of WS due to EDN3 mutation with urinary tract involvement, psychomotor delay and epileptic seizures.

This report aims to show new features in WS caused by EDN3 mutations, that may lead to a deeper understanding of genotype-phenotype correlations and better clinical management at early stage.

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P11.095C

Novel phenotypic and molecular insights in families with Waardenburg syndrome

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Introduction: Waardenburg syndrome (WS) is a type of neurocristopathy. It is clinically and genetically heterogeneous disorder characterized by auditory and pigmentary abnormalities.

Materials and methods: We investigated a cohort of 15 patients from 13 Indian families clinically diagnosed with WS. Candidate gene sequencing for *PAX3* in four families and whole exome sequencing for twelve families was carried out. Extended exome analysis and Real-time PCR were carried out for copy number variant analysis.

Results: Nine novel variants in *PAX3, MITF, SOX10, EDNRB, EDN3* and a reported pathogenic variant in *MIFT* were identified. Intra-familial phenotypic variability was observed in one a family and non-penetrance was observed in two families. We observed low level germline mosaicism in an asymptomatic father of two affected siblings with pathogenic variant, c.256A>T in *PAX3*. Biallelic novel missense variant, c.1021C>G in *MITF* was identified in a patient with WS 2 for the first time in literature. Homozygous c.673G>A in *EDNRB* was identified in a patient without any signs of Hirschsprung disease. Extended

exome analysis for copy number variations revealed 0.17 Mb heterozygous deletion encompassing *SOX10* in a patient with WS 4. Additionally, in two patients fulfilling the diagnostic criteria for WS, we identified a homozygous known stop-gain variant, c.71G>A in *GJB2*, which causes Deafness, autosomal recessive 1A and a novel biallelic stop-gain variant, c.1608C>G in *ADGRV1*, known to cause Usher syndrome 2C.

Conclusion: We herein present novel phenotypic and molecular insights into Waardenburg Syndrome.

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P11.096D

POMK-associated Walker-Warburg Syndrome (WWS) in monozygotic twins with occipital meningocele

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Walker-Warburg syndrome (WWS) represents the most severe end of a phenotypic spectrum of disorders caused by defective glycosylation of alpha-dystroglycan. Alpha-dystroglycanopathies are genetically heterogeneous autosomal recessive disorders; mutations in 16 underlying genes have been identified so far.

We report on monozygotic male twins with strikingly similar manifestations of WWS in prenatal ultrasonography (hydrocephalus, occipital meningocele, hypoplastic cerebellum). Postnatal examination confirmed the meningocele and revealed eye anomalies in both. CK was increased >1000U/l. cMRI scans showed an occipital meningocele with dorsally enlarged fourth ventricle, hypo-/aplasia of cerebellar vermis, cortical malformation with generalized polymicrogyria-like cobblestone malformation, temporo-occipital subcortical band heterotopia, and eye malformations (microphthalmia with coloboma and caudal cyst / persistent hyperplastic primary vitreous body and posterior staphyloma).

Based on clinical signs, elevated CK and radiological findings with brain and eye malformations, the tentative diagnosis WWS was established. Molecular panel analysis revealed a homozygous *nonsense* mutation in *POMK* (OMIM*615247) in both twins. These molecular findings confirmed the diagnosis of a *POMK* associated WWS (OMIM#615247).

So far, with only five different *POMK* mutations published in three families (Jae *et al*., 2013, von Rennesse *et al*. 2014, Di Constanzo *et al*., 2014), *POMK* mutations represent a very rare cause of alpha-dystroglycanopathies. Meningo/encephaloceles have been reported so far as a rare finding in WWS including one patient with *POMK*-related WWS (Jae *et al*., 2013). The observation of occipital meningoceles at identical positions in both twins appears interesting and might point to a more important role of *POMK* in the pathogenesis of neural tube defects.

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P11.097A

Whole exome sequencing in Polish patients as an experience of one Genetic Out-patients Clinic

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Whole exome sequencing (WES) is an universal diagnostic test that can be carried out in relatively short time. WES is commonly used in clinical practice, especially in patients with ambiguous manifestations.

Materials and methods: WES was performed using SureSelect V5 kit and HiSeq1500 sequencing in 107 Polish patients from single Genetic Out-Patients Clinic: 1 foetus, 6 newborns, 10 infants, 84 children and 6 adults. The most common findings were psychomotor development delay, intellectual disability, epilepsy or epileptic encephalopathy, dysmorphic syndromes, athrogryposis, ataxia syndromes, manifestations of neuromuscular disorders, connective tissue diseases as well as central nervous, cardiovascular, urinary, skeletal and eye congenital anomalies. The suspicion of a multigenic syndromes or spectrum of syndromes was established in 78 persons. 29 patients showed highly unspecific clinical symptoms.

Results: Pathogenic mutations consistent with patients' phenotypes were detected in 64 of 107 (60%) cases. The

diagnosis was established in 58 of 78 (74%) patients with clinical suspicion of a multigenic syndromes or spectrum of syndromes and in 16 of 29 (55%) patients with completely unspecific manifestations. Final diagnoses were in accordance with clinical suspicions in 31 of 58 (53%) cases. The most common diagnoses were: 13 cases (12%) - early infantile epileptic encephalopathy, 5 - metabolic defects including mitochondrial disorders, 3 - Schaaf-Yang syndrome, 2 - Joubert syndrome, 2 - GPI, 2- NBIA5, 2 - MICPCH and 2 - spastic paraplegia 47.

Conclusions: In our experience WES proved to be especially useful in patients with infantile onset of non-specific and severely debilitating neurological syndromes.

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P11.098B

Trio whole exome sequencing as an efficient second step strategy to decipher molecular basis of developmental disorders after negative first-tier solo clinical WES

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Purpose: Developmental disorders (DD) and intellectual disability (ID) are frequent disorders affecting around 1-3% of the worldwide population. Genetic defects are estimated to account for approximately 80% of DD/ID etiology, but the diagnosis yield is currently 30-40% when clinical assessment, array-CGH, sequencing of single genes or gene panels and clinical whole exome sequencing (cWES) focusing on known disease-causing genes are combined. In laboratories using solo cWES strategy as a first-tier analysis, a subsequent trio-based strategy is expected to facilitate the selection of candidate variants thanks to rapid identification of *de novo* and compound heterozygous variants, and therefore increase diagnostic yield.

Methods: We selected patients affected by DD/ID who did not get a molecular diagnosis after multiple investigations including solo cWES, and the data were re-analyzed in a trio-based strategy.

Results: Second step trio WES analysis of 53 DD/ID patients selected likely pathogenic variants in 15 individuals (38%) in 12 genes affected by *de novo* variants and 3 genes affected by biallelic variants (11/15 new genes). International datasharing allowed to identify additional patients carrying variants in the same gene with a consistent genotype-phenotype correlation, confirming implication in human phenotypes. Moreover, 5 patients were found to carry a VUS (9%), but datasharing and literature review were insufficient to better classify these variants.

Conclusion: Trio-based WES analysis is a powerful second step strategy for patients affected by DD/ID. It reduces analysis time, limits Sanger sequencing validations, and promises to be an invaluable approach for translational research and identifying new genes.

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P11.099C

Clinical whole exome sequencing and its potential as a diagnostic and disclosing tool

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Whole-exome sequencing (WES) is increasingly used as an effective diagnostic tool for patients with complex phenotypes. Here we report our experience with WES in 282 index patients and assess the result in respect to the rate of molecular diagnosis among phenotypic groups and the ability to introduce novel disease variants and genes. The patients were primarily pediatric (259 [91%] younger than 10 years old; 113 females [40%], 169 males [60%]) demonstrating diverse clinical manifestations, most often including neurological dysfunctions. We could genetically diagnose 122 patients (43%) based on a pathogenic or likely pathogenic variant from which 58 have not been previously reported. These variants are detected in disease genes (*e. g. GLRB, HPS1, ASNS, PLA2G6, SERAC1, MYO18B, PLOD1, SUOX, KMT2D, CASK* and *TBX1*) with significant phenotypic overlap with probands' clinical picture. Given the parental consanguinity of the majority of the examined individuals, recessively inherited phenotypes were most

frequent (73%). However several variants (24%) causative for dominantly inherited diseases such as Kabuki, Cowden, DiGeorge and Coffin-Siris syndrome have been also observed. In further 63 patients a variant of unknown significance was identified. Several new disease candidate genes have been also observed, namely *PTK7* for holoprosencephaly. Our results once again highlight the feasibility and usefulness of the whole-exome sequencing in the clinical setting for timely medical interventions. The high yield of variants from dominant diseases in a largely consanguineous cohort underlines the need for unbiased evaluation and highlights subsequent TRIO analyses as reasonable next step in negative cases.

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P11.100D

Whole genome sequencing as a tool for genetic diagnosis and gene discovery in children with medical complexity

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Children with medical complexity have ≥1 chronic condition(s), functional limitations, multiple subspecialist involvement, and high healthcare utilization. We hypothesized that whole-genome sequencing (WGS) has the potential to efficiently and effectively establish genetic diagnoses for children with medical complexity, and that cohorts of these children are enriched for novel genetic disorders.

Screening 542 children with medical complexity from a single Complex Care Program yielded 126 children (23%) suspected of having an undiagnosed genetic condition despite previous genetic testing. Eligible participants were evaluated through a clinical genetic assessment; WGS was performed in parallel with any outstanding conventional genetic testing.

In the first 21 probands with WGS data (including 13 trios, 1 dyad, and 7 singletons), 11 have reportable primary diagnostic variants. We identified seven pathogenic/likely pathogenic variants in known disease genes, including one small structural variant, one intronic variant predicted to affect splicing, and one mosaic variant; as well as a promising variant of uncertain significance. *De novo* missense variants were also identified in three genes without published disease associations: *FBXW7*, *H3F3B*, and *RAC3*. Importantly, by using public databases (DECIPHER, Matchmaker Exchange, and ClinVar), similarly affected individuals were rapidly identified around the globe and collaborations have been established to publish our joint findings.

Our initial experience applying WGS to children with medical complexity suggests that trio-based genome-wide sequencing is a high yield testing strategy for this patient population, which appears to be enriched for *de novo* mutations and novel genetic disorders. (Funded by University of Toronto and Hospital for Sick Children grants).

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P11.101A

Familial case of Wolf-Hirschhorn syndrome with atypical deletion and asymptomatic carrier

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Introduction: Inheritance of Wolf-Hirschhorn syndrome (WHS) due to familial deletions is reported quite rare. We

describe 4 y.o. boy with mild dysmorphic features, developmental and speech delay and seizures.

Materials and metods, Results: The first performed test with 561-gene customized NGS-platform for the most frequent genes associated with congenital epilepsy using Illumina NextSeq 500 amended to suggest 4p deletion on a coverage basis. Subsequently Microarray analysis with Affymerix Cytoscan Optima array confirmed 1,5 Mb deletion in region 4p without critical WHS region (*NSD2* gene) involvement. The same deletion was also confirmed in proband's phenotypically normal mother. Although her hybridization profile was suggestive for mosaicism, a 4p-subtelomeric FISH in blood samples was performed, but did not confirm this version.

Conclusion: Thus, discrepancy in phenotypes of mother and proband could be explained by possible existence of undescribed imprinted genes in this region or different expression profiles which are to be determined.

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P11.102B
R-SPONDIN2 inhibition of RNF43/ZNRF3 Dictates Limb Numbers Independentlyof LGR4/5/6

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The amphibian *Xenopus tropicalis* is extremely well positioned for modeling human disease. It shares with zebrafish the easy manipulations associated with its external embryonic development, but it manifests unique features making it a more favorable organism. (1) Unlike zebrafish, *Xenopus tropicalis* has a true diploid genome. Hence, gene disruption studies are not suffering from redundancy and (2) its genome shows high synteny with humans, greatly facilitating identification of human disease gene orthologs. We employed a CRISPR/Cas9 based pipe-line for rapid identification of genes influencing limb development and collaborated on a human clinical study on WNT signaling associated amelia. The four R-SPONDIN secreted ligands are considered to act via their cognate LGR4/5/6 and RNF43/ZNRF3 co-receptors to amplify WNT signaling. Here we document an allelic series of recessive *RSPO2* mutations in humans causing Tetra-Amelia Syndrome. CRISPR/Cas9 mediated knockout of *rspo2* in *Xenopus tropicalis* also caused tetra-amelia, confirming earlier findings in mouse knockouts. Unexpectedly, the triple and ubiquitous knockout of *Lgr4*, *Lgr5* and *Lgr6* in mice did not recapitulate the known *Rspo2* LOF phenotypes. Instead, we found that TALEN mediated concurrent deletion of *rnf43* and *znrf3* in *Xenopus* embryos was sufficient to induce super numerous limbs. Our results establish that RSPO2 serves as a direct ligand of RNF43/ZNRF3 but, surprisingly, acts independently of LGR4/5/6. This signal constitutes a master switch that governs limb numbers in the embryo. In conclusion, the combined data clearly demonstrates that the currently accepted paradigm for the LGR-dependent R-SPONDIN signaling pathway is incorrect in the context of limb formation.

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P11.103C
Broader spectrum of OBSL1 mutations

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Case: A 2.5-year-old boy presented with cleft palate, developmental delay and dysmorphic features. He was born term with birth weight of 4242 gram. The cleft palate was seen shortly after birth. Over the years he developed growth retardation, a unilateral conductive hearing loss and a global developmental delay. His facial features showed long eye lashes, broad nasal bridge, vertical line in lower lip, dental problems, prominent ears with broad and bifid earlobes, micrognathia. He also had fleshy hands and feet and flat feet. He is the second child of a Polish father and Thai mother. Family history is non-contributory.

DNA testing: A 'congenital anomaly' gene panel showed compound heterozygosity for variants of unknown significance (missense) in the OBSL1 gene. Both parents are carriers. The variants have not been described before. At an earlier stage DNA testing for Kabuki syndrome did not show any abnormalities.

Discussion: OBSL1 gene mutations are involved in 3M syndrome type 2. 3M syndrome is characterized with extremely short stature, skeletal abnormalities and in general normal intelligence. Our patient, however, does not have extremely short stature and does not show radiological findings seen in 3M syndrome. Additionally, our patient has a developmental delay. The question rose if these variants are causing this boy's symptoms. Are OBSL1 gene mutations causing a broader spectrum than skeletal problems? Are there other patients with a similar phenotype?

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P12 Cancer genetics

P12.001A
rs2910164 in miR-146a-3p is associated with increased mortality in thyroid cancer patients

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Papillary thyroid carcinoma (PTC), the most common thyroid cancer subtype, is considered a disease of low mortality. Still, a significant number of patients die within few years from the disease onset, mainly due to the resistance to post-operative radioiodine treatment. There is a need for markers allowing for stratification of low and high-risk PTC patients.

In this study we analyzed impact of the germline rs2910164 polymorphism in miR-146a-3p on the overall survival of PTC patients and on the expression of the sodium-iodide transporter NIS.

The study included 2441 patients (2163 women, 278 men), including 359 cases with follicular variant of papillary thyroid carcinoma (fvPTC). Tumor/blood DNA was used for rs2910164 genotyping. Overall survival was assessed retrospectively (median follow–up 10.25 years). The miR:*NIS i*nteractions were analyzed using *in vivo* and radioactive iodine uptake assays.

Rs2910164 functional variant within miR-146a-3p is associated with increased overall mortality among fvPTC female patients. The deaths per 1000-person-years were 29.7 in CC vs. 5.08 in GG/GC-carriers (HR=6.21, *P=0.006*). Higher mortality of CC vs. GG/GC carriers was also observed in patients with lower clinical stage (HR=22.72,$P < 0.001$), smaller tumor (HR=25.05, *P<0.001*), lack of extrathyroidal (HR=9.03,*P*=0.02) and nodular (HR=7.84,*P=0.002*) invasion, lack of metastases (HR=6.5,*P*=0.005) and older (HR=7.8,*P=0.002*). We also showed that miR-146a-3p directly regulates *NIS*. Inhibition of mir-146a-3p restores the expression and function of *NIS*, increasing radioactive iodine uptake.

We propose a novel molecular marker of the clinical outcome of PTCfv patients. Rs2910164 increases the overall mortality with inhibition of NIS and disruption of radioiodine uptake as a possible mechanism.

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P12.002B
A novel translocation of t(2;14)(q31;q32) in B-cell precursor acute lymphoblastic leukemia

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Introduction: Cytogenetics is among most useful tools of predicting prognosis and choosing appropriate therapy for haematological diseases. Translocations of 14q32 where

IGH gene resides, are found approximately 5% of acute lymphoblastic leukemias. Among this group of translocations, t(2;14)(q31;q32) with B-cell precursor acute lymphoblastic leukemia has not been reported in literature so far.

Materials and methods: Here we present a 27 years old female patient who referred to our clinic with diagnosis of B-cell precursor acute lymphoblastic leukemia. GRAALL-2003 protocol initiated after evaluation. During chemotherapy, she developed mucormycosis in nasal region. Fournier's gangrene developed at 36th day of the patient's admission and debridement surgery with colostomy performed immediately.

Results: Karyotype from bone marrow resulted as:46,XX [6]/46,XX,t(2;14)(q31;q32)[9], FISH analysis from same sample revealed signal patterns consistent with IGH rearrangement in 50% of interphases. Probes targeted to cMYC, p16 del, MLL and BCR/ABL showed no abnormal signal. RT-PCR of BCR/ABL was negative. Histopathological and flow cytometry from bone marrow were consistent with B-cell precursor acute lymphoblastic leukemia.

Conclusion: The patient died a day after debridement surgery due to respiratory failure.

Considering detrimental clinical course, short survival and age of this patient, it seems reasonable that this translocation is concordant with the putative knowledge of 14q32 translocations occuring at an early age and leading poor prognosis. In view of being first case reported, we hope this case would be a contribution to literature and a further step for comprehension of mechanisms lying behind this disease and ultimately, to discovery of an effective therapy for patients affected.

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P12.005A

Molecular mutations, their cooccurrences & prognostic value in acute myeloid leukemia

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The aim of the research was to analyze the prognostic effect of typical for AML patients mutations.

Materials and methods: The study included 620 patients from Germany and Russia. Screening of mutations in genes *FLT3*, *NPM1, DNMT3A, IDH1/2* was performed by PCR and sequencing.

Results: Mutations were found in 343/620 (55.3%) patients and were more often detected in patients with normal karyotype ($p = 0.001$). The presence of *FLT3*-ITD mutation was associated with adverse overall survival (OS) and relapse-free survival (RFS) ($p = 0.005$ and $p = 0.009$, respectively). Increasing *FLT3*-ITD allelic ratio (≥0.5) correlated with low OS ($p = 0.028$). Patients with single *NPM1* mutation reached significantly better OS and RFS compared to other patients ($p = 0.040$, $p = 0.049$, respectively). The negative influence (tendency) of *DNMT3A* mutations and *IDH1* polymorphism rs11554137 was found ($p = 0.112$, $p = 0.186$, respectively), whereas the presence of *IDH1* mutations correlated with better outcome compared to group without mutations ($p = 0.092$). In 144 patients various combinations of mutations (from 2 to 5) were detected. The presence of 2 mutations in one patient significantly reduced OS compared to patients with one mutation ($p = 0.003$). The worst prognosis was for patients with combinations of *NPM1+/FLT3-ITD+*, *NPM1+/FLT3-ITD+/DNMT3A+*, *DNMT3A+/FLT3-ITD+* mutations.

Conclusions: Mutations in analyzed genes are frequent in intermediate risk group of AML patients. They significantly affect the prognosis, wherein it is important to consider the type of mutation, its allele ratio and the presence of additional mutations. Complex analysis of genetic aberrations in AML patients provides the most accurate prognosis prediction and planning of targeted therapy.

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P12.006B

NIPBL a new player with NPMc+ in the onset of acute myeloid leukemia

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Cohesins form a multimeric protein complex (SMC1A, SMC3, RAD21, STAG and additional proteins NIPBL, MAU2, ESCO1, HDAC8) involved in the cohesion of sister chromatids, post-replicative DNA repair and transcriptional regulation. Recently, recurrent somatic mutations and deletions of cohesins have been reported in the 10% of the patients with Acute Myeloid Leukemia (AML) or other myeloid neoplasms. Frequently, mutations in cohesin genes co-occurred with the known AML-associated gene *nucleophosmin* (*NPM1*) that, when mutated, aberrantly relocates to the cytoplasm (NPMc+). Forced NPMc+ expression in zebrafish embryos causes an expansion of hematopoietic stem cells (HSCs) according with AML patient features.

In our cohort of adult AML patients, we observed a specific and significative reduction of the *NIPBL* expression in NPMc+ patients. We generated a zebrafish model of *nipblb* haploinsufficiency to investigate the hematopoietic phenotype and the interactions between NPMc+ and *nipblb*. In *nipblb*-loss-of-function zebrafish embryos, we observed an increase in myeloid progenitors, a phenotype resembling the NPMc+ zebrafish model. Therefore, we characterize the functional interaction between NPMc+ and *NIPBL* in the onset of the aberrant hematopoietic phenotype in zebrafish and showed the involvement of the canonical Wnt pathway in this process.

We demonstrate for the first time a role for *NIPBL* during zebrafish hematopoiesis and that its decreased expression, due to *NPM1* mutations, might play a role in leukemia onset.

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P12.007C

Genomic and transcriptional profiling of acute myeloid leukemia by next-generation sequencing unravels patient-specific patterns of post-transplantation relapse

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Introduction: allogeneic hematopoietic stem-cell transplantation represents the most effective treatment for patients with high-risk Acute Myeloid Leukemia, but post-transplant relapses remain frequent. Next generation sequencing allows the investigation of relapse mechanisms, but tumor heterogeneity, clonality and genomic complexity demands for ad-hoc bioinformatics solutions.

Methods: we combined whole exome sequencing and RNA-Seq to characterize AML samples collected and purified from 14 patients before (preTx) and after allo-HSCT (postTx). We used alt-aware aligners to efficiently map reads to hg38DH reference, and alignment-free quantification of RNA-Seq data to detect differentially regulated transcripts. Somatic variant-call was performed both on DNA/RNA sequencing data.

Results: we detected an average of 15 damaging somatic mutations per leukemic sample, a total of 54 exclusive to relapses. The mutational burden increased significantly from pre-to-postTx ($p = 0.02$, Wilcoxon) and consistently, clonal-analysis evidenced the emergence of new clones at relapse in 8 patients. Relapse-specific mutations encompassed known AML driver-genes including WT1 and KRAS but no gene evidently related to immune function. By linear-model analysis of RNA-Seq data we found ~500 genes significantly deregulated in blasts at postTx-relapse. In contrast to the genomic data, most of these genes belong to immune-related categories, including antigen-processing and presentation via HLA Class II and T cell costimulation pathways.

Conclusions: postTx-relapses originate upon a complex process, in which leukemia clonal evolution intertwines with immune-driven changes in patient-specific combinations, on which precision treatments should be tailored.

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P12.008D

No association between rs1534309 gene polymorphism and FLT3 ITD mutation in patients with acute myeloid leukemia

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Introduction: Rs1534309, one of the polymorphism of minichromosome maintenance complex component 7

(MCM7) gene, was reported to be associated with several types of cancer. The aim of our study was to evaluate if there are any correlation between the mentioned polymorphism, the risk of developing acute myeloid leukemia (AML),FLT3 ITD mutation status, overall survival and cytogenetic risk.

Material and Methods: A number of 143 patients and 375 healthy people were enrolled in the study. The FLT3 ITD status was determined by RFLP-PCR and HRM techniques. ABI 7500 fast real-time PCR system and TaqMan assay were used for rs1534309 genotyping.

Results: For rs1534309 we found the following genotypes: 86 wild type in patients group and 252 in control group,52 heterozygous in patients group and 114 in control group, 5 homozygous with the variant allele in patients group and 9 in control group. In patients group a number of 23 patients were positive for FLT3 ITD mutation (14 were wild type for rs1534309, 8 heterozygous and 1 homozygous with the variant allele). No associations were found between this polymorphism, AML risk and FLT3 ITD status. No significant differences were detected between sex,ages, overall survival, cytogenetic risk or demographic characteristics and genotypes.

Conclusion: For our population rs1534309 polymorphism is not a risk factor for AML, is not associated with FLT3 ITD mutation and overall survival in AML patients. Acknowledgement:This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation,CNCS/CCCDI-UEFISCDI,project number PN-III-P2-2.1- PED-2016-1076 within PNCDI III, contract no.147PED/2017.

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P12.009A

No association between TERT rs2853669 polymorphism and NPM1, DNMT3A gene mutations and acute myeloid leukemia risk

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Introduction: Telomerase reverse transcriptase gene (*TERT*) which is important for the maintenance of chromosome stability and it was reported to be significantly associated with different cancer types, including malignant hemopathies. We investigated the association of a functional single nucleotide polymorphism (SNP), namely rs2853669, in the gene that encodes telomerase reverse transcriptase (TERT) with the risk of acute myeloid leukemia in a Romanian population.

Materials and methods: One hundred and forty-three AML patients, and one hundred seventy-three healthy subjects with no history of any malignancy were included in the present case-control study.

Results: No association between variant allele of the TERT rs 2853669 and AML risk was observed (OR = 1.55, p = 0.71). We noticed a slightly shorter overall survival in the cases with homozygous variant genotypes compared to those with wild type homozygous genotype (p = 0.048). In addition, we investigated the relation of variant genotype of mentioned SNP and NPM1 and DNMT3A mutations in AML cases but no association was observed. In conclusion, we consider that TERT rs2853669 SNP is not a risk factor for the development of AML in our cohort.

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P12.010B

Integrating multiple genetic analysis into the clinical diagnosis of Acute Myeloid Leukemia: a case report

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Introduction: Testing for genetic markers is strongly recommended by all international guidelines as an essential step for proper diagnosis, prognosis and subsequent monitoring of acute leukemias. In the absence of genetic lesions the decision-making process might become difficult. We report a case of Acute Myeloid Leukemia (AML) in a young adult in which genetic analysis, performed at diagnosis according to European LeukemiaNet, showed no alterations.

Methods: Conventional cytogenetic analysis, fluorescence in situ hybridization (FISH), molecular assays as polymerase chain reaction (PCR) amplification and fragments analysis, Next Generation Sequencing (NGS) and array comparative genomic hybridization (CGH) were

applied for the identification of genetic lesions in leukemia cells.

Results: Cytogenetic analysis was unsuccessful and FISH assay of *TP53* and *KMT2A* provided normal results. Molecular evaluation of *RUNX1-RUNX1T1, CBFB-MYH11, BCR-ABL1* fusion genes and *NPM1, CEBPA, FLT3* mutations, proved negative. NGS analysis suggested a duplication of *TET2* and *KIT* genes and a partial deletion of *RUNX1*. Array CGH results confirmed a trisomy of chromosome 4 and a partial deletion of chromosome 21 where the above genes are mapped. In addition, a partial tetrasomy of chromosome 13 and a partial deletion of chromosome 17 were identified. The treatment of the patient was modified accordingly.

Conclusions: The laboratory evaluation of leukemias is complex and has evolved significantly with the incorporation of advanced techniques. The combination of multiple genetic approaches helped identifying prognostic markers leading to proper risk category stratification and a better patient management.

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P12.011C

The Effects of Metformin on the PI3K/AKT Pathway in Anaplastic Thyroid Cancer Cell Lines

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Introduction: Association between T2D with the thyroid cancers was shown and it has been attributed to the insulin resistance. Insulin has relevance to tumor growth and metastasis and it can exert its cell growth-stimulating effects via PI3K/AKT signaling pathway. The aim of the present study was to indicate whether metformin could affect insulin-promoting cell growth by regulation of the PI3K/AKT pathway.

Material and Methods: Anaplastic thyroid cancer (ATC)-derived cells were treated with 0-60 mM metformin for 24, 48 and 72h. Cell viability, morphology, apoptosis, and migration were investigated by MTT assay, microscopy observation, AnexinV-PI and wound healing assay, respectively. The mRNA levels of PI3K and AKT were evaluated using qRTPCR. The phosphorylated levels of PI3K and AKT were determined by ELISA.

Results: Metformin inhibited the cells proliferation and migration in a significant time- and dose-dependent manner. It induced apoptosis and caused morphological changes in all examined cells. qRTPCR results showed that the PI3K and AKT mRNA levels were inhibited by metformin ($P<0.05$). There was no change in the mRNA level of AKT following metformin treatment in C643 cell line ($P>0.05$). The ELISA results showed that metformin treatment had no effects on the phosphorylated levels of PI3K and AKT ($P>0.05$).

Conclusuions: The inhibition of proliferation by metformin strongly was associated with the downregulation of the molecules involved in the PI3K/AKT pathway. The exact molecular mechanism of the metformin on the inhibition of PI3K/AKT pathway and subsequent suppression of cell proliferation has remained unclear and further studies are required to its clarification.

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P12.012D

Transcriptional profiling reveals a potent activity of anticancer imidazoacridinone C-1311 against prostate cancer cells expressing androgen receptor

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Introduction: The androgen receptor (AR) plays critical role in the poor response of androgen-dependent and castrate-resistant prostate cancers to chemotherapy. Anticancer imidazoarcidinone C-1311 (Symadex™) is a new inhibitor of topisomerase II and receptor tyrosine kinase FLT3 that has been tested in phase II studies against metastatic breast cancer. Here, we assessed the effect of C-1311 on the transcriptional profiles in prostate cancer cell lines with different AR status.

Materials and Methods: Prostate cancer cells were exposed to C-1311 for 24 h. Global transcriptome profiling for LNCaP (AR+) and DU-145 (AR-) cells was performed

using Illumina Hiseq 4000 platform. The biological relevance of dysregulated transcripts was assessed by Ingenuity Pathway Analysis (IPA) software.

Results: IPA analysis of transcripts uniquely expressed in LNCaP and DU-145 cells exposed to C-1311 revealed activation of diverse signalling pathways depending on AR status. The main enriched top canonical pathways identified in LNCaP (AR+) cells were associated with multiple cell cycle control and survival processing genes like ATM and breast cancer signalling whereas glycolysis, gluconeogenesis and lipid metabolism pathways were predominantly dysregulated in DU-145 (AR-) cells. Furthermore, the main upstream regulators and their target genes in both cell lines were involved in the different biological processes.

Conclusion: Taken together, our finding suggest that AR status may significantly affect the efficacy of C-1311 against prostate cancer cells. Association of specific canonical pathways indicates the opposite mechanism of action of imidazoacridinone C-1311 in both androgen-dependent and independent prostate cancer. Founded by the National Science Centre, Grant No 2013/09/D/NZ7/04185.

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P12.013A

O1 *($CuFeO_2$)* and O2 *(Cu_2O)* Nanoparticles (NPs) induced cytogenetic and genotoxic effects on human cultured lymphocytes, antimicrobial activity on three bacterial strains and damaging effects on pDNA

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<META NAME="author" CONTENT="Χρήστης των Windows">

Introduction: O1 *($CuFeO_2$)* and O2 *(Cu_2O)* nanoparticles (NPs) have been tested at various concentrations in human peripheral blood cells *in vitro* in order to investigate their genotoxic, cytotoxic and cytostatic effects. DNA damaging action was estimated by the Sister Chromatid Exchanges (SCEs) methodology, a method for controlling genotoxicity of human exposure to different mutagenic agents and by DNA electrophoretic mobility experiments on pDNA (pUC18). Their antimicrobial activity was evaluated against the Gram-positive bacterial strains *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 11778), and *Staphylococcus aureus* ATCC 29213, and the Gram-negative *Escherichia coli* (XL1), *Xanthomonas campestris* (ATCC 33013).

Results: These NPs at various concentrations demonstrated high and significant genotoxic, cytostatic and cytotoxic effects while the frequency of SCEs/cell was increased 4-6 times over the control level. Strong antibacterial activity of O2 was also observed against all the bacterial strains and furthermore the effect of increasing concentrations of the newly synthesized NPs on the integrity and electrophoretic mobility of pDNA showed that both NPs mimic topoisomerase I enzymatic nicking activity.

Conclusion: These results suggest that the O1 and O2 NPs exert strong genotoxic, cytogenetic, antimicrobial and pDNA damaging activity, providing a significant property that might support its possible involvement in DNA damaging phenomena and their possible clinical use as a new potent anticancer agent.

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P12.014B

Heterogeneity of *KRAS*, *NRAS*, *PIK3CA* and *BRAF* mutational status in primary tumor, lymph nodes and liver metastases obtained from patients with metastatic colorectal cancer

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It is well known that activating mutations in the *KRAS* and *NRAS* genes are associated with poor response to anti-EGFR therapies in patients with metastatic colorectal cancer (mCRC). Approximately half of the patients with wild-type *KRAS* colorectal carcinoma do not respond to these therapies. This could be because the treatment decision is determined by the mutational profile of the primary tumor, regardless of the presence of small tumor subclones harboring RAS mutations in lymph nodes or liver metastases. We analyzed the mutational profile of the *KRAS*, *NRAS*, *BRAF* and *PI3KCA* genes in samples of 26 paired primary tumors, 16 lymph nodes and 34 liver metastases from 26 untreated mCRC patients. The most frequent mutations found in primary tumors were *KRAS* and *PI3KCA*, followed by *NRAS* and *BRAF*. The distribution of the mutations in the 16 lymph node metastases analyzed was as follows: 4 in *KRAS* gene, 3 in *NRAS* gene and 1 mutation each in PI3KCA and BRAF. The most prevalent mutation in liver

metastasis was in the *KRAS* gene, followed by *PI3KCA* and *BRAF*. Of the 26 cases studied, 15 displayed an overall concordance in the mutation status detected in the lymph node metastases and liver metastases compared with primary tumor, suggesting no clonal evolution. The mutation profiles differed in the primary tumor and lymph node/metastases samples of the remaining 11 patients, suggesting intertumoral clonal evolution. Our results suggest the need to perform mutational analysis in all available tumor samples of patients before deciding to commence anti-EGFR treatment.

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P12.015C
BAP1 germline variants in Australia

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Introduction: Germline mutations in BAP1 are linked to a spectrum of cancers, defined as the BAP1 tumour predisposition syndrome (BAP1-TPDS). The most commonly associated cancers are uveal melanoma (UM), mesothelioma, cutaneous melanoma, renal cell carcinoma, cholangiocarcinoma and meningioma, however, there is likely a 'long tail' of rare cancers also associated with the syndrome.

Materials and Methods: To assess the prevalence of BAP1-TPDS in Australia, we screened Queensland UM probands for BAP1 mutations (n = 64), identified variants from published studies from Australia, and liaised with national clinical genetics services to ascertain families with BAP1 germline variants. Variants with a population frequency of <0.0005 were considered for a potential role in BAP1-TPDS.

Results: We identified 4 truncating variants, all in kindreds with classical features of BAP1-TPDS. Of the 11 missense variants, only 1 (p.T173C), is from a family with typical BAP1-TPDS features, and is predicted to impair ubiquitin hydrolase activity. In silico prediction suggests 8/9 unique missense variants either alter splicing or damage protein structure, though all require functional confirmation. A promoter variant identical to that reported in an Italian family with BAP1-TPDS was observed, however, in the Australian family, incomplete family history for the proband did not allow for assessment of cancer history.

Conclusions: Detection of four definite and two likely deleterious variants in this focused assessment of BAP1 in Australia suggests a need for a comprehensive worldwide database of germline BAP1 variants and cancers present in carriers and development of refined guidelines for clinical testing.

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P12.016D
ZNF384 gene fusions in BCP-ALL: A report of nine Austrian cases secured by systematic FISH and array screening

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The subdivision of childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL), based on specific genetic features, such as gene fusions or ploidy and copy number aberrations, provides the basis for treatment stratification and decisions. The so-called "B-other" group embraces all cases with rare recurrent abnormalities that are hitherto less well-defined. Because they include potential candidates for targeted and personalized therapies, they are currently the main focus of interest. One of these recently identified subgroups that accounts for approximately 4% of BCP-ALL and up to 10% of B-other cases, involves the *ZNF384* gene, which is fused to at least ten different partners. These cases have commonly a CD10 negative (pro-B/BI) or $CD10_{low}$ immunophenotype with myeloid markers and a distinct gene expression pattern. To search for *ZNF384* positive cases we screened all B-other cases that were enrolled in the ALL-BFM 2009 study with SNP/CGH arrays as well as an additional selected cohort with a *ZNF384*-specific dual color break apart FISH probe set. We found nine patients with a *ZNF384* fusion, which make-up approximately 5% of all B-other cases. Five of them had a *EP300-ZNF384* and two a *TCF3-ZNF384* fusion. The remaining two had novel fusion partners, one of which was ascertained as *CCAR1*. The other one will be hopefully identified with whole transcriptome RNA-sequencing, which is currently performed in all cases. Three of them had *IKZF1* deletions and

all but one are in remission, supporting the notion that *ZNF384* positive cases seem to respond well to current therapies.

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P12.017A

Development of *BCR-ABL1* positive chronic myeloid leukemia in a patient with *JAK2* V617F positive polycythemia vera

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Introduction: The *BCR-ABL1* oncogenic fusion gene is a hallmark of chronic myeloid leukemia (CML). Polycythemia vera (PV) belong to a *BCR-ABL1* negative myeloproliferative neoplasm. Transformation of PV to CML has been rarely reported. Here, we present a secondary CML case progressed from long-standing *JAK2* V617F positive PV.

Materials and Methods: A 68-year-old woman presented with easy bruising. Complete blood count (CBC) showed elevated hemoglobin level (hemoglobin concentration, 17.9 g/dL), leukocytosis (white blood cell count, 28.48 x 10^9/L), and marked thrombocytosis (platelet count, 1779 x 10^9/L). Bone marrow was hypercellular with a marked myeloid and megakaryocytic hyperplasia.

Results: Allele-specific polymerase chain reaction (PCR) revealed heterozygous *JAK2* V617F mutation and reverse-transcriptase (RT)-PCR was negative for *BCR-ABL1* rearrangement. G-banding showed normal karyotype. The patient was diagnosed with PV and treated with hydroxyurea. Seven years following diagnosis, CBC revealed re-occurrence of neutrophilia and thrombocytosis, and newly developed basophilia (16% of white blood cells; basophil count, 5.45 x 10^9/L). G-banding revealed Philadelphia chromosome in all analyzed metaphases. RT-PCR showed positivity for *BCR-ABL1* transcript and the patient was diagnosed with CML. The patient has been followed with imatinib and hydroxyurea.

Conclusions: In conclusion, we report a case of secondary *BCR-ABL1* positive CML progressed from *JAK2* V617F positive PV. Although the development of CML in PV is a rare event, careful examination including molecular studies should be considered in patient with PV who showed severe leukocytosis and/or basophilia.

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P12.019C

Expression patterns analyses of TCGA datasets reveal new interactions and regulatory factors of immune response in bladder cancer

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Cancer immunotherapy is becoming increasingly popular in both research and clinical medicine. The potential to harness the patient's immune system to selectively destroy cancer cells is promising in theory, and indeed already yielded some impressive results in the clinic. However, the individual success rate of such therapies is still very low. Many patients do not respond to treatment, while a small percentage of others suffer from catastrophic side effects. The immune response is mediated by immunological synapse: an interface between lymphocytes and antigen presenting cells (APCs), which consists of co-inhibitory and co-stimulatory checkpoint proteins. Therefore, a better understanding of immunological synapse will enable us to better design clinical trials and will provide us with better diagnostics and therapeutics.

In this study we focus on The Cancer Genome Atlas expression datasets and computational analyses to reveal the regulatory patterns linking checkpoint genes to miRNA or other regulatory factors that might enhance or switch off their activity.

The method can be generally applied to all types of cancer with sufficient expression datasets. To showcase this method, we have applied it to a bladder cancer (BLCA) cohort. The graphical Gaussian model shows co-expression of several checkpoint genes and anti-correlated expression profile of many miRNAs, most prominently *mir-15a* and *miR-15b*. This model predicts miRNA as negative regulators of immunological response, potentially serving as a post-transcriptional regulator of checkpoint genes expression. We have validated these interactions using the calculation of free-energy of miRNA-mRNA binding, and independently in cell lines using qPCR expression profiling.

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P12.020D

Distrubition of BRAF gene mutations in the patients with malignant melanoma

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The BRAF gene encodes a protein with serine/threonine kinase activity involved in the mitogen activated protein kinase signaling pathway (MAPs). It causes cell proliferation through the ras pathway. Somatic BRAF gene mutations were detected in 80% melanomas, 15% colorectal cancers(CRC), 45% papillary thyroid cancers(PLT) and 15% nonsmall cell lung cancers(NSCLC). Mutations are frequently observed in the codon 600 (V600A, V600D, V600E and V600K,R,M), exons 15 and 11. The advent of therapeutics targeting the MAPK signal pathway has led to great advances in the treatment of metastatic melanoma.

This study was designed to investigate the mutation status of 11th and 15th exons of BRAF gene in 83 melanoma patients. Sequence analysis from paraffin embedded tissue samples was performed by pyrosequencing method The overall incidence of somatic mutations within the BRAF gene was 37,35%. The clinical subtypes of the melanomas were compared with the mutation types and ratio. The most prevalent type of BRAF mutation was c.1799T>A (27,71%). According to the literature, about 40-60% of melanoma patients have mutations. The findings of our study were supported the literature. The detected mutations considered as potential drug targets in advanced melanoma therapy.

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P12.022B

Assessing the effectiveness of the Manchester Scoring System in predicting Southeast Asian patients with *BRCA1/2*germline mutations

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Background: Results from *BRCA1/2* germline mutation testing are important in guiding clinicians on cancer management and surveillance strategies. The Manchester scoring system (MSS) helps identify patients indicated for germline *BRCA1/2* testing. The recent third iteration of MSS showed improvements after including further adjustments for triple negative breast cancer, high grade serous ovarian cancer and HER2 receptor status. This study evaluates the relative effectiveness of MSS1-3 in a Southeast Asian population.

Methods. We carried out a retrospective study of 330 index patients that were genetically tested using next generation sequencing (NGS) panels which included full gene sequencing and coverage for large deletions/duplications in *BRCA1/2*. Clinicopathological features (e.g., tumour histology, grade and age of diagnosis) and family cancer history were collected to calculate MSS1-3 scores and evaluated against genetic results with ROC analysis. Calculations were performed using Medcalc17.

Results In total, 47 (14.2%) patients were tested positive for *BRCA1* or *BRCA2* germline mutation. Based on a 10% likelihood of *BRCA1/2* germline mutation as a threshold for genetic testing, 43.0% (142/330) of patients were indicated for testing under MSS3, compared to 35.8% (118/330) for MSS1 and 36.4% (120/330) for MSS2. In terms of model effectiveness, MSS3 had a statistically significant improvement over MSS1 ($p = 0.037$) and MSS2 ($p = 0.032$), with 91.5% sensitivity and 65.0% specificity at the 10% threshold.

Conclusion. Compared to previous iterations, the latest MSS3 is a better performing model and relative to the United Kingdom population, is equally effective in distinguishing patients with *BRCA1/2* germline mutations in a Southeast Asian population.

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P12.023C

Detection of *BRCA1* and *BRCA2* variants in circulating free DNA by using a commercial kit

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Introduction: Patients with high-grade serous ovarian cancer with germline or somatic mutations in *BRCA1/2* genes can benefit from PARP inhibitors treatment. While germline mutational screening has become a routine practice by Next Generation Sequencing, detection of somatic mutation is challenging. The major advantage of using liquid biopsy, rather than tissue biopsy as tool for mutation screening in cancer, is to scan allele status and frequencies of a patient over time. In this preliminary study, we demonstrated that detection of both germline and somatic *BRCA1/2* mutations in circulating free DNA (cfDNA) with targeted amplicon sequencing is feasible and might be helpful in the standard diagnostic procedures.

Materials and Methods: cfDNA of patients, previously screened for *BRCA1/2* mutations, was extracted using QIAamp Circulating Nucleic Acid Kit, while *BRCA1/2* mutation screening was performed using the Devyser BRCA kit. In addition, two known reference standards (BRCA Somatic Multiplex I FFPE and Structural Multiplex cfDNA Reference Standard) carrying different pathogenic and not pathogenic variants with known percentage of mutated allele were analyzed.

Results: We confirmed the presence of all germline mutations in cfDNA and the entire set of variants with known percentage of mutated alleles in the two reference standards achieving a sensitivity as low as 5% for somatic variants.

Conclusions: Our results demonstrate that cfDNA mutation screening with a commercial kit allows the detection of both germline and somatic *BRCA1/2* mutations at the same time, permitting to monitor the presence of possible reverse mutations, which could affect the efficacy of PARP inhibitors. GRANT: RICATEN2017

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P12.024D

Factors involved in decision to undergo risk reduction bilateral salpingo-oophorectomy in carriers of BRCA mutations

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The lifetime risk of ovarian cancer among BRCA1,2 mutation carriers is 36 times higher than general population (50% vs. 1.4%, respectively), and risk of breast cancer is increased by 5.8 (70% vs 12%, respectively). Risk-Reducing-Bilateral-Salpingo-Oophorectomy (RRBSO) is widely recommended for mutation carriers by professional organizations (NCCN, NCI etc.). RR-BSO reduces ovarian cancer by 95%, and decreases all-cause mortality. Various aspects influence decision-making-process regarding surgery. By understanding these considerations, we could increase RRBSO-rate.

Objective: to estimate RRBSO-rate among BRCA mutation carriers, and examine effect of demographic factors and cancer history on RRBSO-rate

Methods: Data on 543 female carriers of BRCA1/2 mutations from age 35 were collected from medical records and attendance to our specialized-high-risk-clinic . Chi-squared, Fisher-exact tests and student-t-test were used for categorical, continuous variables, respectively.

Results: Overall RRBSO-rate was 83.5% . There was positive correlation between mean age and RRBSO-rate: 53.27(± 9.66)yrs. in those with RR-BSO vs. 44.17(± 8.56)yrs. in those without, $p < 0.001$. Parous carriers were more inclined to have RR-BSO than nulliparas: 84.5% vs. 61.5%, respectively, $p = 0.045$. These results are consistent with common medical recommendations based on age, birth-planning. No association was found between marrital status and RRBSO. There was positive correlation between breast cancer and RRBSO: 92.2% with positive history, vs. 81% without, $p = 0.033$. Familial cancer history had no significant impact.

Conclusions: the vast majority of BRCA mutation carriers that are attend our specialized-high-risk-clinic, choose to undergo RRBSO, in line with acceptable medical recommendations. Age, parity, and personal history of breast cancer are positive predictors of RRBSO.

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P12.026B

Development of a dual platform strategy for targeted DNA sequencing in genetic screening

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The use of next-generation sequencing (NGS) to assess genetic variation in genes involved in both inherited diseases, as well as cancer, is critical. However, obstacles including homology to pseudogenes and difficult to sequence motifs arise, making genes like CFTR, BRCA1, and BRCA2 challenging to sequence with short-read (SR) chemistry. Long-read (LR) assays overcome these issues,

but are restricted by limited available technologies. To enhance the benefits of each platform, we developed targeted, single-tube, multiplexed amplicon assays that allow for overlapping, contiguous coverage.

DNA from MUTCF-2 (Coriell Institute) was used in both the SR and LR assays. In the SR assay, an 87-amplicon panel comprehensively covered all exons (~10Kb) in CFTR. The LR assay covered all the same exons, and 6 complete intronic regions, using 21 amplicons (~54Kb). DNA from BC01 (Coriell Institute) was used in the 246-amplicon (~23Kb) SR assay and the 35-amplicon LR assay (~86kB). While both comprehensively cover all exons, the LR assay additionally covers 20 complete introns. For all genes, SR libraries were sequenced on an Illumina MiniSeq and LR libraries were sequenced on a Pacific Biosciences RSII. Greater than 95% on-target and coverage uniformity was seen in libraries from both SR and LR assays, and the same number of variants were detected.

SR assays allow for screening of a wide range of targets for immediate and broad use. LR assays enable comprehensive sequencing of entire gene coding regions and neighboring introns, gaining additional insight into variants in difficult-to-sequence regions, large insertion/deletions, and structural variations.

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P12.027C

Still not 100%: A 15 year evaluation of genetic counselling rates for serous ovarian cancer at a single Canadian cancer centre

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Introduction: An estimated 15-20% of invasive serous ovarian cancers (SOC) are related to pathogenic variants in *BRCA1/2*. Guidelines recommend that all women with SOC are offered genetic counselling (GC) irrespective of family history or age at diagnosis. Despite this, most centers report suboptimal referral rates. Prior to 2009, only 23% of women with SOC were seen for GC at Princess Margaret Cancer Center (PM) in Toronto, Canada. While some centres have approached this issue with automatic referrals or oncologist-mediated genetic testing, PM implemented changes to pathology reporting, appointment scheduling, and in-service education for referring physicians.

Methods: Following the implementation of new referral and scheduling processes, a list of women diagnosed with SOC at PM between 2010-2016 was obtained from the PM Cancer Registry and cross-referenced against the genetics database.

Results: Of 724 women with SOC, 68% were referred for GC and 93% attended their scheduled appointment. Of those who received GC, 96% proceeded with genetic testing, 22% of whom were found to have a pathogenic variant in *BRCA1/2*. Notably, 25% of women with a pathogenic variant reported no family history of breast or ovarian cancer.

Conclusion: Despite substantial improvements in referral rates, many eligible women are not receiving GC. Considering the high rate of pathogenic variants, therapeutic implications, and the value of identifying high-risk families, it is imperative that all women with SOC are offered genetic testing, irrespective of family history or age at diagnosis. Our study demonstrates that even simple interventions can address barriers to GC.

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P12.028D

Features of male breast cancer: Review from a single Canadian hereditary cancer clinic

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Introduction: Male breast cancer (MBC) accounts for <1% of breast cancers cases, with an incidence of ~230/year in Canada. The limited data available on MBC suggests that ~18% are hereditary, that most are diagnosed with advanced (stage 3-4) disease, and are typically ER/PR+, Her2-. No studies have examined Canadian MBC characteristics. A better understanding of the genetic and pathologic profiles of MBC would improve risk assessment and genetic counselling.

Methods: A retrospective chart review examined the characteristics of males diagnosed with MBC between 2000 and 2017, and who received genetic testing at Princess Margaret Cancer Centre.

Results: A total of 29 men were identified; 20 received *BRCA1/2* genetic testing and 9 received multi-gene panel testing (MGPT). No pathogenic variants were identified in

BRCA1/2; however, 1 *CHEK2* pathogenic variant was detected. The average age of diagnosis was 64.1 years. The majority of men were diagnosed with invasive ductal carcinoma (86%), of which 55% were stage 1. 100% of cancers were ER+, 83% PR+, and 96% Her2-. 62% of men reported a family history of breast and/or ovarian cancer, 21% a second non-breast primary, and 7% a second breast primary.

Conclusions: Our data supports the current literature that most MBC are ER/PR+ and HER2-. In contrast, our study found an earlier stage of cancer and a relatively low mutation rate. The absence of *BRCA1/2* mutations in this unselected Canadian cohort of MBC demonstrates the role of MGPT to increase the diagnostic yield of genetic testing.

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P12.029A

Functional evaluation of variants of unknown significance in the homologous recombination genes *BARD1* and *BRCA2*

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With the advent of personalized medicine, genetic testing has become increasingly prevalent. Detection of pathogenic variants facilitates enhanced screening measures, early intervention and modified treatments. Unfortunately, a significant portion of the genetic testing results are the non-actionable variants of unknown significance (VUS). Functional studies specific to the altered functional domains are needed to ascertain their pathogenicity. Here, we functionally assessed the pathogenicity of *BARD1* and *BRCA2* clinical VUS identified among young colorectal cancer patients.

BARD1 and *BRCA2* harbor domains for homologous recombination via RAD51 nuclear localization while *BARD1* has additional apoptotic domains. RAD51 nuclear localization was assessed using nuclear-cytoplasmic fractions of patient-derived lymphoblastoid cells upon induction of DNA breaks. Apoptotic function was determined by immunoblotting of activated p53 and downstream marker, activated caspase-3.

Two *BARD1* and one *BRCA2* VUS were tested. DNA repair was unaffected in *BARD1* variants, evident from the normal RAD51 nuclear localization and previous homology-directed repair studies. However, their apoptotic function was impaired with failure of caspase-3 activation upon induction of apoptosis. This correlated well with their mutation sites which were proximal to apoptotic domains of ankyrin and BRCT repeats respectively. For *BRCA2* variants, homologous recombination was impaired for *BRCA2* c.9154C>T but not *BRCA2* c.440A>G.

To facilitate in reclassification of variants, RAD51 nuclear localization assay can be performed on homologous recombination VUS. For *BARD1*, the right assay should be chosen based on the affected functional domain - DNA repair or apoptosis. Inappropriate use of the assays may misinform the variant's pathogenic status.

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P12.031C

Germline loss-of-function variants in the *BARD1* gene are associated with familial breast cancer

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Introduction: Recent studies revealed a weak association of *BARD1* germline loss-of-function (LoF) variants with breast cancer (BC). We determined the *BARD1* mutation prevalence in n = 3,348 well-characterized BC index patients of the German descent and geographically-matched female control individuals (GMCs; n = 2,196).

Methods: Female BC index patients and GMCs were screened for LoF variants in the *BARD1* gene by next generation sequencing. All patients met the inclusion criteria of the German Consortium for Hereditary Breast and Ovarian Cancer for germline testing. The SOPHiA DDM® platform (SOPHiA GENETICS®) was applied for the detection of copy number variations (CNVs) in a subset of 2,810 BC patients.

Results: We identified 14 LoF variants (excluding CNVs) in 3,348 female BC index patients (carrier frequency [CF]:0.42%) compared with 36 in a total of 36,694 controls (CF:0.10%, OR=4.276, 95%CI=2.30-7.94, p = 0.00004). The CF in each control dataset was 0.07% (GMCs; n = 2,196), 0.11% (FLOSSIES; n = 7,325) and 0.10% (ExAC; n = 27,173), respectively. The *BARD1* p.Gln564* variant was found in 6/14 patients. CNVs affecting the *BARD1* gene were identified in 3/2,810 BC index patients

(CNV CF=0.11%), which is higher than that observed in the FLOSSIES database (2/7,325; CF=0.03%). For the 17 *BARD1* mutation carriers, the mean age of first BC diagnosis was 46 years (range 24-60) compared with 47 years (range 17-92) in the overall sample (n = 3,348).

Conclusion: We observed a significant association of deleterious *BARD1* variants with the BC phenotype and confirm *BARD1* as a moderately-penetrant risk gene. The inclusion of CNVs is crucial for a comprehensive genetic screening of BC patients.

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P12.032D

Experience learned from *BRCA1* and *BRCA2* screening in Iranian women

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Breast cancer is the most common malignancy and second leading cause of cancer-related death among women. 5-10% of breast cancer cases are hereditary and several associated loci have been identified. *BRCA1* and *BRCA2* genes are the most important genes involved in breast cancer. Here we investigated *BRCA1* and *BRCA2* genes in 240 women with clinical presentations of breast cancer (n = 141) or asymptomatic females with positive family history of this cancer (n = 99). All exons plus flanking regions were enriched and sequencing was performed by Illumina platform. Classification of detected variants was done based on ACMG guideline for variant interpretation 2015. 52 samples out of 240 samples (21.6%) have at least one VUS (Variant of Uncertain Significance), likely pathogenic, or pathogenic variant. Among these 52 variants, 13 were pathogenic, 9 were likely pathogenic and 30 variants were VUS. 23 variants were found in *BRCA1* and 29 in *BRCA2* gene. 36 of 52 variants were detected in symptomatic and 16 variants in asymptomatic women. Among 52 detected variants, 29 were missense, 16 frameshift, 4 nonsense, and 3 splicing variants. Full data and analysis will be presented in the meeting.

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P12.033A

Importance of verifying the location of duplicated sequences - example of a benign complex duplication in the *BRCA1* gene in a breast cancer patient

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Approximately 24% of breast (BC) and/or ovarian cancer (OC) cases who met the inclusion criteria of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) for germline testing are caused by pathogenic mutations in the *BRCA1* and *BRCA2* gene. About 1% of patients carry deleterious CNVs (copy number variations) in *BRCA1* or *BRCA2*. According to the guidelines of the GC-HBOC, confirmed CNVs of every size are estimated as pathogenic, if they result in a frameshift and thereby disrupt functional protein domains. While the pathogenicity of deletions is less complicated to determine, the situation for duplications is much more complex, because duplications can appear either intragenic or extragenic. Here, we present a case (BC at age 26) carrying a complex duplication in *BRCA1*. Initial MLPA analysis showed a duplication of exon 1-8 and 11-12 of *BRCA1*. RNA and molecular combing experiments could not confirm an intragenic duplication but gave evidence for a translocation of the duplicated sequence. The patient is carrying a pathogenic mutation in *PALB2* as well (c.654del, p.(Asp219Thrfs*4)), which is most probably the cause of the BC disease. This is also supported by the fact that her healthy mother (57) carries only the *BRCA1* variant and not the *PALB2* mutation. The majority of duplications appear in tandem, but this case shows the great importance of verifying this assumption. When the duplicated fragment is translocated elsewhere in the genome, the mutation is most likely not pathogenic, which has a great impact on clinical consequences and predictive testing of relatives.

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P12.034B

BRCA1 and BRCA2 mutational spectrum in breast cancer patients from the Republic of Macedonia

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Identification of the spectrum of mutations in *BRCA1* and *BRCA2* genes in specific populations allows for implementation of a cost-effective genetic screening. Thus far, the *BRCA1/2* mutations present among the BC patients from the Republic of Macedonia have been largely unknown. Therefore, we used targeted next-generation sequencing, Sanger DNA sequencing and multiplex ligation probe amplification analysis to search for point mutations and deletions/duplications involving *BRCA1* and *BRCA2*-coding regions. We have analyzed a total of 337 BC patients, enriched for family history of cancer, early age of onset and bilateral and/or triple negative BC. A total of 28 pathogenic mutations were observed in 55 unrelated BC patients (55/337, 16.3%) with the predominance of *BRCA2* mutations (29/55, 52.7%). Nine novel mutations were identified; four in *BRCA1* and five in *BRCA2*. The most prevalent mutations were c.181T>G, c.1102G>T, c.3700_3704del5, c.5212G>A and c.5266dupC in *BRCA1* and c.5722_5723delCT, c.5851_5854delAGTT, c.7879A>T and c.8317_8330del14 in *BRCA2* gene. Thus far, *BRCA2* c.7879A>T and c.8317_8330del14 mutations have been described in several isolated cases however, our study is the first one showing that they have a founder effect among Macedonian population. Nine recurrent mutations account for 63.6% of all of the detected mutations allowing for implementation of a fast first-step *BRCA1/2* mutational screening strategy. In conclusion, this study provides a comprehensive view of known and novel *BRCA1/2* mutations in BC patients from the Republic of Macedonia and contributes to the global spectrum of *BRCA1/2* mutations in BC.

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P12.035C

Next Generation sequencing based detection of copy number variations in hereditary breast- and ovarian cancer germline diagnostics

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Introduction: Next Generation Sequencing (NGS)-based detection of copy number variations (CNVs) is widely used in a routine diagnostic setting. The prevalence of CNVs in *BRCA1/2* and other risk genes in patients with familial breast cancer (BC) or ovarian cancer (OC) remains elusive.

Methods: We used the TruRisk® gene panel (Agilent SureSelect) which covers 34 cancer risk genes/candidate risk genes for BC/OC. The Sophia Genetics DDM platform was employed to predict CNVs. conspicuous findings were verified by MLPA.

Results: A cohort of 4,418 index patients (3,846 BC, 445 OC, and 127 BC/OC patients) was included. All cases met the inclusion criteria of the German Consortium Hereditary Breast- and Ovarian cancer for germline testing. Most CNVs predicted by DDM platform were detected in the *BRCA1*, *BRCA2* and *CHEK2* genes. CNVs were also observed in further (candidate) cancer predisposition genes *ATM*, *BARD1*, *FANCA*, *MLH1*, *MSH2*, *PALB2*, *PMS2*, *RAD50*, *RAD51C*, *RAD51D*, *TP53*, but not in *MSH6*, *CDH1*, *NBN*, *FANCM*, *PTEN*, *STK11*. For *BRCA1*, 43 CNVs were predicted in 4,418 patients, of which 40 (93%) could be confirmed by MLPA. For *BRCA2*, 9 CNVs were predicted, of which 5 could be confirmed (56%). In total, about 1% of all index patients carried CNVs affecting the *BRCA1/2* genes.

Conclusions: In summary, bioinformatic analysis of NGS gene panel data detects CNVs. However, it remains to be determined whether sensitivity/specificity of *in silico* CNV detection meets diagnostic criteria.

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P12.036D

Identification of differential genes in luminal breast tumors by comparison of Brazilian and American population data

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Introduction: Luminal breast tumors are the most common type of diagnosed breast cancers, having worse prognosis in the long-term. Chemotherapy is less effective in this group, which makes research in this field necessary. The objective of this study was to compare data from Brazilian and American populations of luminal breast carcinomas to evaluate the differences in the amplified or deleted genes between these sets of samples.

Subjects and Methods: 49 samples of luminal breast tumors from Brazilian women were selected by histopathological characterization of biopsy specimens. We performed the chromosome microarray (CMA) technique to assess Copy Number Variations (CNVs) and indels from these tumors. Raw data was segmented by PSCBS followed by normalization. Copy number aberration (CNA) analysis was performed using GISTIC2. The Brazilian genes data found to be significantly amplified or deleted were compared to TCGA data obtained for American samples.

Results: We found significantly amplified or deleted genes that belong mainly to the FGF and Wnt signaling pathways exclusively in the cohort of Brazilian breast tumors.

Conclusions: These data suggest that there are differences between Brazilian and American luminal breast tumors at the genomic level, potentially affecting tumor biology, influencing their prognosis and response to treatment. This comparison should bring light to the question of population differences in terms of genomics, personal habits and the environment, having an effect on tumor genetics leading to resistance to the available treatments.

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P12.038B

Constitutive promoter methylation analysis of breast cancer associated genes in women with isolated early onset breast cancer

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Introduction: Early age at onset of breast cancer (eoBC) is considered to be suggestive of an increased genetic risk. Although genetic testing of known high penetrance genes is offered to all eoBC-affected women, in the absence of a positive family history the detection rate of pathogenic variants is <10%. This study aimed at assessing the role of constitutive promoter methylation at BC-associated loci as an underlying predisposing event in women with eoBC and negative family history.

Materials and methods: Promoter methylation at 12 loci (*BRCA1*, *BRCA2*, *ATM*, *CDH1*, *FANCM*, *STK11*, *NBN*, *PALB2*, *PTEN*, *RAD51C*, *RECQL* and *TP53*) was assessed by the EpiTYPER-MassARRAY assay in blood from 110 *BRCA1/2* negative patients with eoBC and negative family history, and 60 healthy donors (controls). Hypermethylation was determined, within each promoter, by comparing the patient's mean methylation value with thresholds based on the upper limit of the 95% bootstrap confidence interval of the controls' mean.

Results: Only one patient, with mean methylation value of 26%, exceeds the threshold by 0.15 at the *BRCA1* promoter. Interestingly, analyses on FFPE BC from the patient reveal a mean *BRCA1* methylation of 60-70% and the loss of the unmethylated allele. Another patient, with mean methylation level of 21%, exceeds the threshold by 0.05 at the *RAD51C* promoter, though its biological significance is yet to be defined.

Conclusions: In isolated eoBC patients, *BRCA1* constitutive promoter methylation appears to be an underlying predisposing event. Further studies are required to define the impact of slight methylation changes involving other BC-predisposing genes.

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P12.039C

Large case-control study and functional analyses show that FANCM truncating mutations are associated with a breast cancer risk magnitude that varies depending on their location

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Breast cancer (BC) is the most common female oncological disease. About 50% of the familial cases are explained by rare mutations in *BRCA1* and *BRCA2* and other high-risk genes, by mutations in moderate-risk genes including *PALB2*, *ATM* and *CHEK2*, and by common low-risk alleles. Previously, other and we showed that truncating mutations within the *FANCM* gene are associated with BC risk, particularly with ER-negative subtype. Also, we recently observed that upstream mutations cause more severe clinical phenotypes than those located in the C-terminus.

In this study, the three most common *FANCM* truncating mutations p.Arg658*, p.Gln1701* and p.Arg1931* were genotyped in 67,112 BC cases and 53,776 controls collected within the Breast Cancer Association Consortium. Logistic regression analyses indicated that the p.Arg658* is a moderate risk factor for ER-negative and triple negative BC (TNBC): OR=2.44 (1.12-5.34) and OR=3.79 (1.56-9.18), respectively. Similar analyses showed that the p.Gln1701* and p.Arg1931* may confer a lower risk in TNBC [OR=2.15 (1.05-4.38)] and ER-negative BC [OR=1.98 (1.26-3.13)], respectively.

We are now testing these and other two patient-derived truncating mutations subjecting transfected human *FANCM*$^{-/-}$ cells to DNA-ICL inducing agents to measure survival rates and chromosome fragility. Consistently with our genetic and clinical data, our initial functional results support the hypothesis that upstream FANCM truncation could be associated with a higher BC risk and more severe clinical phenotypes. These results will allow a better BC risk estimate in *FANCM*-mutation carriers from families and general population.

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P12.040D
Germ line gene panel analysis in a HBOC population

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Introduction: The Belgium core gene panel for analysis of Hereditary breast and ovarian cancer (HBOC) patients consists of 4 genes *BRCA1*, *BRCA2*, *PALB2*, *TP53*, along with the c.1100delC mutation in *CHECK2* and inspection for copy number variations in *BRCA1/2*. Patients are selected according to national guidelines (www.BeSHG.be). Broadening the analysis with an identification of mutations in the Lynch genes *MLH1*, *MSH2* and *MSH6*, and screening for truncating mutations in the *ATM*, *BRIP1*, *RAD51C/D* genes has been proposed recently.

Methods: Mutation analysis was performed with the BRCA Hereditary Cancer MASTR Plus kit (Multiplicom). Deletion/duplication analysis was performed with the MLPA kits P002-D1 and P045-C1 (MRC Holland). The presence of reported variants is confirmed with Sanger sequencing.

Results: A retrospective data analysis of the fastq files for the gene pool proposal was performed for 700 patient samples previously analyzed. An additional 13 families were identified, with the following diversification: 6 *ATM*, 3 *BRIP1*, 1 *RAD51C*, 1 *RAD51D*, 1 *MLH1* and 1 *MSH6* gene families. These patients were all negative for class 4 and 5 core gene panel variants.

Conclusion: Broadening of the present core gene panel, as proposed, will result in the identification of only a limited extra number (1.85%) of patients with germ line mutations as the possible molecular cause of their cancer. However, this molecular diagnosis can have major influences on the treatment and follow-up of the index cases, while it also offers possibilities for predictive testing and family planning in at risk family members.

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P12.041A
Identification of ultra-rare loss of function variants in a west of Ireland breast cancer population

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Breast cancer is the most common female cancer globally. Approximately 5-10% of breast cancers are due to inherited variants in numerous breast cancer susceptibility genes, of which 20-30% are in *BRCA1/BRCA2*. Variants in other genes demonstrate reduced penetrance. The genetic heterogeneity of breast cancer means that next-generation sequencing(NGS) using multi-gene panels is a cost- and time-efficient way to identify causative germline variants. We aimed to use NGS to identify pathogenic variants contributing to breast cancer susceptibility in an Irish population. A multi-gene panel was designed on a Roche-Nimblegen platform. Sequencing was performed on an Illumina NextSeq. GATK best practices (2016) were followed for bioinformatics analysis. Samples were confirmed to be unrelated using Plink1.9; population stratification using 1000 Genomes data confirmed ethnicity. Variants in 90 patients with breast cancer and 68 unaffected controls were annotated using Snpeff, VEP, and Annovar. Conflicting annotation was clarified by manual interpretation via UCSC genome browser. One loss-of-function(LOF) variant was identified in *BRCA1*. Ultra-rare variants (MAF $\leq 1.5 \times 10^{-5}$) were identified in genes frequently appearing on breast cancer risk panels($n = 3$); genes implicated in breast cancer susceptibility in GWAS ($n = 2$); genes reported to be somatically mutated/hypermethylated in breast cancers ($n = 2$). Pathogenic LOF variants were identified in nine other genes including *RECQL4, OBSCN, TTN, NOTCH3*. Our results show that NGS increases diagnostic yield, but also increases the yield of variants in genes with weak/unconfirmed association with breast cancer. Further analysis is required to determine if these variants are incidental findings, or causal variants.

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P12.042B

Multiple germline mutations in breast cancer patients-useful?

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The use of multigene "panels" has become common in hereditary cancer testing, the result being more fortuitous findings, variants of unknown significance (VUS) and "low risk" mutations, which may not be clinically relevant. We report here the experience of our multidisciplinary breast team with 128 breast cancer patients having the same 17-gene panel. Our philosophy thusfar has been to initiate most testing with BRCA 1-2, followed by reflex testing to a panel; this approach will underestimate the number of patients harboring more than one mutation. The finding of multiple germline mutations in a few patients has led us to reflect on the relative contribution of the mutations to their clinical phenotypes and to risk assessment strategies for their families.

Results: Two patients had BRCA mutations only (panel testing done because of family history). Six patients had mutations in other panel genes: ATM, BRIP1, PALB2, CHEK2. 18% of patients had a VUS in one or two genes. Two young patients with metastatic invasive ductal carcinoma at diagnosis (grade 3, ER/PR-positive, Her-2 negative), neither of whom had a close family history of cancer, had multiple mutated genes. Patient #1, age 37, had a synchronous renal cell carcinoma. She had germline mutations in BARD-1, CHEK-2, and PALB2. Patient #2, age 23, has Neurofibromatosis type I, without prior significant medical complications. She was found to have mutations in both the NF-1 and MUTYH genes. As literature on such cases is scant, multicenter reporting is needed to develop management strategies for such patients.

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P12.043C

Hereditary breast cancer gene variants- multigene panel testing outcome from Sri Lanka

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Introduction: Globally one in eight women develops a breast cancer (BC) in her lifetime. As per the national statistics, 38.7% Sri Lankan women aged 39-46 years account for BC which is ranked number one among all cancers. About 5–10% of BCs are clustered in families owing to germline mutations. In the era of Next Generation Sequencing (NGS), a panel based genetic testing for hereditary BC is feasible and cost effective.

Materials and Methodology: Credence Genomics provides NGS based screening for inherited predisposition to BC. A multi gene panel consists of 18 genes, including *BRCA1, BRCA2, TP53, ATM, BARD1, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, NF1, PAL B2, PTEN, RAD50, RAD51C, RAD51D* and *STK11* are incorporated in the panel. A descriptive, retrospective study was carried out from July 2014–December 2017 of all the patients referred

for hereditary BC screening at Credence Genomics laboratory.

Results: A total of 53 patients either who have been affected or with a family history of BC were included to the study. Pathogenic mutations were detected in 6(11%) patients of which two were affected with BC and three had a strong family history of BC. Four patients had *BRCA1* gene variants(*p*.Arg1203Ter, *p*.Glu907Ter, *p*.Leu28Argfs and *p*.Glu23Valfs), one had *BRCA2* gene variant(*p*. Phe12Leufs), one had *TP53* gene variant(*p*.Arg248Gln). One patient had a benign deletion in *BARD1* gene(*p*. Leu359_Pro365del).

Conclusions: Of the pathogenic variants detected, *p*. Leu28Argfs mutation has been previously reported in Asian patients. There is no reliable data for the incidence of the other mutations in the Asian population.

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P12.044D

Analysis of gene rearrangements in the BRCA1 and BRCA2genes in patients with suspected hereditary breast and ovarian cancer syndrome in the Brazil-central region

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The present study aimed to identify the prevalence of gene rearrangements in *BRCA1/2* in patients diagnosed with breast cancer in central Brazil. We evaluated 47 patients with breast cancer who met the criteria of the National Health Agency published in the document "Annex II: guidelines for use to cover supplementary health procedures" for research on HBOC syndrome. A 4mL blood sample was collected for DNA extraction using a commercial kit and the MLPA (Multiplex Ligation dependent Probe Amplification) technique was performed using the SALSA MLPA P002 BRCA1 and SALSA MLPA P045 BRCA2 / CHECK2 kits. The majority of the patients were female (95.75%) and the mean age of the patients was 39 years. The most common molecular subtype was luminal A (50%) followed by triple negative tumors (27.27%). No patients were found with BRCA1 gene rearrangements. In BRCA2, one patient (2.12%) presented deletion in heterozygosity of the exon 27, being this female, with HER2 superexpressor tumor diagnosed at 24 years old and with a family history of prostate and stomach cancer. We can conclude that the frequency of gene rearrangements in the Central-Brazilian population is low.

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P12.045A

Digenic inheritance of *RASSF1A* and *KLK3* mutations in an Iranian multiple-case breast cancer family

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Introduction: Much of the hereditary breast cancer risk in families is still unexplained. Additional breast cancer susceptibility genes might be detectable via exome sequencing in multiple-case breast cancer families.

Methods: To elucidate the molecular basis of breast cancer in the Iranian population, we carried out exome sequencing of genomic DNA from an Iranian patient with a strong family history of breast cancer (4 first-degree family members affected) but negative for *BRCA1* and *BRCA2* mutations. We then investigated specific mutations in twelve other members of this family. Furthermore, we directly genotyped specific mutations in a breast cancer case-control series from Iran.

Results: Exome sequencing identified novel mutations with predicted pathogenicity in the three candidate genes *KLK3*, *RASSF1A*, and *FAM81B*. The *KLK3* truncating mutation segregated with breast cancer in the family. The *KLK3* gene product, PSA, has been implicated in both breast and prostate cancer. However, a supportive segregation pattern was also observed for a novel missense mutation in *RASSF1A* (p.S135F). This mutation eliminates the ATM phosphorylation site on the RASSF1A protein, a known tumor suppressor in breast carcinomas. The truncating mutation in *FAM81B* did not segregate with breast cancer in this family. The *KLK3* and *RASSF1A* mutations were not observed in further 235 Iranian breast cancer patients and 260 controls.

Conclusions: Our findings illustrate the difficulties to identify the causal gene if candidate mutations are restricted to the single family and show a similarly plausible segregation pattern. Digenic or oligogenic inheritance may contribute to breast cancer risk in such cases.

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P12.046B

Exome sequencing and case-control analyses identify *RCC1* as a candidate breast cancer susceptibility gene

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Introduction: Breast cancer is a genetic disease but the known genes explain a minority of cases. RCC1, the Regulator of Chromosome Condensation 1, is important for replication control and proper mitotic spindle formation but has not previously been associated with hereditary cancer.

Methods: To elucidate the molecular basis of breast cancer in the Tunisian population, we performed exome sequencing on six BRCA1/BRCA2 mutation-negative patients with familial breast cancer. We then directly genotyped a Tunisian breast cancer case-control series for the identified *RCC1* mutation, and further analysed tumors from six mutation carriers for loss of heterozygosity.

Results: Exome sequencing identified a novel frameshift mutation *RCC1**c.1067_1086del19. Subsequent genotyping detected the 19-bp deletion in additional 5 out of 153 (3%) breast cancer patients but in none of 400 female controls ($p = 0.0015$). The deletion was enriched in patients with a positive family history (5%, $p = 0.0009$) and co-segregated with breast cancer in the initial pedigree. The mutant allele was lost in 4/6 breast tumors from mutation carriers which may be consistent with the hypothesis that RCC1 dysfunction provides a selective disadvantage at the stage of tumor progression.

Conclusions: The results suggest *RCC1* as a novel breast cancer susceptibility gene and encourage further search for germline *RCC1* mutations in cancer patients from other populations. Grant reference: This work was funded by the German Ministry of Education and Research and the Tunisian Ministry for Higher Education and Scientific Research (TUNGER-70).

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P12.047C

Identification of variants predisposing to breast cancer through a WES approach

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Introduction: The two major genes BRCA1 and BRCA2, together with rare high-penetrance genes (p53, PTEN, CDH1, STK11) and moderate-penetrance genes such as PALB2 collectively explain ~30% of familial Breast Cancer (BC) risk. Expanding our knowledge to additional genes is crucial to extend the benefits of targeted surveillance/prevention to a larger population of high-risk women.

Materials and methods: Whole Exome Sequencing (WES) was performed on constitutional DNA with Nextera coding exome library enrichment and annotated using an in-house pipeline. Mutation screening was performed via TruSeq Custom Amplicon panel and VariantStudio. Expression studies were performed on MCF10A and MCF7 breast cell lines.

Results: WES on first-degree affected cousin-pairs identified detrimental variants in *ROS1*, *RASAL1, POLN*, and *NPL* genes. Analysis with a target custom-made kit in 131 unrelated patients with familial and/or early onset BC who had tested negative for BRCA1 and 2 revealed rare/novel variants with a significant allele frequency difference between cases and controls ($p < 10^{-3}$), in particular in ROS1. MCF10A cells expressed ROS1 mRNA, whereas MCF7 cells did not, suggesting that ROS1 absence may be correlated to tumor development. Moreover, a ROS1 splice-site variant, detected in two affected cousins and in one unrelated patient, resulted in altered splicing, demonstrated via minigene approach, and inserted a premature stop-codon in the protein.

Conclusion: We identified novel/rare damaging variants in genes not previously associated to BC risk. Replication in independent samples and further functional analysis are ongoing to elucidate their role in BC development. Supported by Italian Ministry of Health- grant DIANE to EB.

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P12.048D

Important Effect of BIBR1532 and Rapamycin Combination on Breast Cancer Stem Cells

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Introduction: Cancer stem cells (CSCs) have features of self-renewal, proliferation, differentiation similar to normal stem cells. Targeting CSCs has been considered as a new approach in cancer therapy. The stemness and replicative properties of CSCs are related to telomerase activity. BIBR1532 has been used as a quite effective inhibitor of hTERT. It is known that mTOR regulates telomerase activity at the translational and post-translational level. PI3K/Akt/mTOR pathway is common in breast cancer and the interaction between the mTOR pathway and hTERT is important for the survival of cancer cells.

Materials and Methods: We performed cell culture in this study. The WST-1 solution was used to determine the cytotoxicity of BIBR1532. IC_{50} doses of Rapamycin and BIBR1532 on BCSCs were calculated via CalcuSyn Version 2.0 software Annexin-FITC Detection Kit was used for apoptosis, Cycletest Plus DNA Reagent Kit was used for cell cycle. RNA Isolation was performed and Real-time PCR was used for gene expression analysis (Qiagen). Furthermore, hTERT gene expression was investigated.

Results: The IC_{50} doses of Rapamycin and BIBR1532 were detected as 7.87 nM and 23 μM, respectively for in 48^{th} hour on BCSCs. The combination was increased apoptosis 4.79 fold compared to control. We observed significant changes in expression levels of mTOR related genes. BIBR1532 reduced hTERT activity compared to control. In addition, Rapamycin and BIBR1532 further reduced hTERT activity compared to only BIBR1532 treatment.

Conclusion: Consequently we show that treatment of BIBR1532 and rapamycin is effective on mTOR pathway, moreover, it is important to target to BCSCs.

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P12.049A

Computational Variant Impact Prediction for Gain-of-Function Somatic Missense SNVs

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The majority of human mutations that cause cancer are somatic missense single nucleotide variants (smSNVs), and tumors evolve through positive selection on somatic gain-of-function (GoF) smSNVs in oncogenes (OGs) and loss-of-function (LoF) smSNVs in tumor suppressor genes (TSGs). Computational variant impact prediction (CVIP) tools such as SIFT, PolyPhen, LINSIGHT, CADD and FATHMM can classify SNVs as benign or deleterious, where deleteriousness suggests an alteration to protein structure or function. We sought to determine if CVIP can distinguish between GoF and LoF SNVs, including both smSNVs and germline mSNVs (gmSNVs). In order to answer this question, a database of $n = 28{,}744$ GoF and LoF smSNVs in $n = 530$ cancer causing genes (CCGs) from COSMIC were scored using FATHMM and CADD. A linear relationship was observed between scores (R^2=0.05, $p < 1x10^{-10}$), and CADD scores were lower (less deleterious) for variants predicted "CANCER" by FATHMM ($p < 1x10^{-10}$). A similar trend was observed in $n = 7{,}664{,}768$ gmSNVs, where CADD scores were lower in CCGs (17.0 ± 0.05) versus all genes (18.1 ± 0.07; $p < 10^{-10}$). Scores were higher for gmSNVs in TSGs (19.7 ± 0.2) versus OGs (18.7 ± 0.2; $p < 10^{-10}$). These results suggest that CVIP produces higher "deleteriousness" scores for LoF SNVs than for GoF SNVs, which can lead to inaccurate classification of causative GoF SNVs in cancer.

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P12.050B

Identification by a multi-omic approach of new biomarkers indicative of a constitutional defect in the *TP53* and *BRCA* tumor suppressor genes

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The identification of the constitutional mutation responsible for a genetic predisposition to cancer is essential to the clinical management of the patient and its relatives. With the implementation of high-throughput sequencing to the diagnostic routine of these pathologies, the challenge no longer lies in the detection of alterations but in their biological and clinical interpretation. While specific treatments are emerging, simple functional assays to help with the interpretation of the detected variants are needed. In this context, we are currently evaluating the relevance of a

multi-omic approach combining high-throughput analysis of the transcriptome and the metabolome to identify new biomarkers able to discriminate cells with a deleterious heterozygous mutation from wild-type cells. Using genotoxic drugs, we exacerbated the differences between the lymphocytes of 4 control subjects, 4 Li-Fraumeni patients carrying a deleterious mutation in the *TP53* gene and 4 patients carrying mutations in the *BRCA* genes involved in hereditary breast and ovarian cancers. We performed a total RNA-Seq experiment to analyze both coding and non-coding RNA transcripts. This analysis revealed a list of genes differentially expressed between wild-type and mutant conditions. In parallel, we will perform non-targeted analysis of the metabolome (UHPLC-IM-MS) of these cells. The data will then be integrated to target the key pathways and biological actors of these cellular responses. The identified biomarkers will be integrated to simple functional tests fitted to the diagnostic routine. This work is supported by the Ligue contre le Cancer, the Canceropôle Nord-Ouest (Emerging Project), the Normandy Region and Europe (ERDF)

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P12.052D

Germline mutations in DNA repair genes predispose asbestos-exposed patients to malignant pleural mesothelioma

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Introduction: A direct correlation between the amount of asbestos exposure and the risk of malignant pleural mesothelioma (MPM) is apparent, but not all individuals exposed to high level of asbestos develop MPM. This observation and the reports of families with multiple MPM cases suggest a role for inherited predisposition. *BAP1* tumor predisposition syndrome (TPDS) includes mesothelioma in its tumor constellation, but we found *BAP1* mutations in only 3/28 MPM probands with familial MPM. We hypothesized that genes involved in other TPDS could also predispose to MPM.

Materials and Methods: We investigated the prevalence of germline variants in 94 cancer-predisposing genes in 93 MPM patients with a quantified asbestos exposure using NGS.

Results: Ten pathogenic truncating variants (PTVs) were identified in *PALB2, BRCA1, FANCI, ATM, SLX4, BRCA2, FANCC, FANCF, PMS1* and *XPC*, all genes involved in DNA repair. Mutated patients (9.7%) had a significantly lower asbestos exposure than non-mutated patients ($p = 0.0015$). This result remains significant ($p < 0.0001$), when four patients carrying *BAP1* germline mutations are included in the analysis.

Conclusions: These data suggest that patients with germline mutations in DNA repair genes show increased susceptibility to asbestos-induced MPM. Our preliminary findings (Betti 2017) preceeded a paper (Robinson 2017), that reported PTVs in 12.2% of 500 patients with metastatic tumors (75% in DNA repair genes). Therefore, our observation likely reflects a general phenomenon of carcinogenesis. According to the concept of BRCAness, patients with germline mutations in homologous recombination repair genes may respond to drugs that induce synthetic lethality. Grants: AIRC 2015-IG17464(GM), IIGM(GM), ISS2013-14(CM).

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P12.053A

Detection of oncogenes hotspot mutations in female NSCLC tumor DNA and cell-free DNA

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Objectives: The aim of this study was to assess non-small cell lung cancer (NSCLC) female patients' hotspot mutations in oncogenes and compare it between formalin-fixed, paraffin-embedded (FFPE) tumor DNA and plasma cfDNA samples.

Materials and Methods: 46 female patients with NSCLC were included in the study. 15 (33%) patients were heavy smokers. The dominant morphology was adenocarcinoma - 33 (72%). Hotspot mutations in 22 oncogenes *(KRAS, EGFR, BRAF, PIK3CA, AKT1, ERBB2, PTEN, NRAS, STK11, MAP2K1, ALK, DDR2, CTNNB1, MET, TP53, SMAD4, FBX7, FGFR3, NOTCH1, ERBB4, EGFR1, FGFR2)* were detected using NGS (Ion Torrent™ PGM) Ion AmpliSeq colon and lung cancer research panel (ThermoFisher). NGS was performed from FFPE DNA and plasma cfDNA for every patient. Samples were taken before treatment.

Results: Mutations in *EGFR*, *KRAS*, *TP53*, *ALK* and *MET* genes were identified most frequently. Mutations were observed in 5 (11%) females tumor and plasma samples. For 2 females mutations were detected only in plasma sample and for 27 (59%) females only in tumor samples. The remaining 12 (26%) females had no mutations detected in both types of samples. Deletion, insertion and duplication in *EGFR* and *ERBB2* genes were detected in 11 tumor samples, however, only one deletion in *EGFR* gene was detected in plasma sample.

Conclusion: The mutations were detected in 32 (70%) tumor samples and in 7 (15%) plasma samples. Detection of mutations using plasma cfDNA samples could be only an additional assay in a routine clinical work.

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P12.055C
Breast cancer in Slovene *CHEK2* mutation carriers

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Introduction: Pathogenic variants in *CHEK2* which encodes a cell-cycle-checkpoint kinase are associated with moderate risk of developing breast cancer (BC). We collected and analysed data on clinical characteristics, family history and mutation spectrum in Slovene *CHEK2* mutation carriers with BC.

Materials and Methods: 25 BC patients from 21 different families who developed 29 tumours and were verified or obligatory *CHEK2* mutation carriers were included in our analysis. Clinical information was obtained from their medical records and family pedigrees.

Results: We detected four recurrent *CHEK2* pathogenic variants in our families: c.444+1G>A (23.8%), c.349A>G (23.8%), c.1100delC (18.2%) and deletion of exons 9-10 (13.6%). 62.5% of our patients had a first or second degree relative with BC and 87.5% had a first or second degree relative with any cancer. A male patient developed oestrogen receptor (ER)-positive, progesterone receptor (PR)-positive and human epidermoid growth factor receptor (HER2)-positive invasive ductal carcinoma (IDC) at the age of 49. Of the 24 female patients, four developed bilateral BC. Median age of onset for female BC patients was 42.5 (range 21-64). 22.7% of their cancers had lobular histology, the rest being IDCs. 91.1% were ER-positive, 81.8% were PR-positive, 22.7% were HER2-positive and 9% were triple negative.

Conclusions: *CHEK2* mutations seem to be associated with ER- and PR-positive disease, family history of cancer and early age of disease onset in Slovene BC patients. Although our analysis is severely limited by small sample size and ascertainment bias, our findings are compatible with what is currently known about *CHEK2*-positive BC patients.

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P12.056D
Co-existence of multiple subclones in ETV6/RUNX1 at diagnosis of B-cell lymphoblastic leukemia

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Introduction: The chromosomal translocation t(12;21)(p13;q22) which results in the formation of the ETV6/RUNX1 fusion gene is the most common structural genetic aberration in childhood B-cell lymphoblastic leukemia (B-

ALL). Co-existence of multiple subclones in ETV6/RUNX1 is rare with limited clinical information available. Herein is described a case of B-ALL with multiple subclones in ETV6/RUNX1.

Clinical Report: The patient, 9-year-old-boy, was admitted to the Department of Pediatric Haematology at Pamukkale University Hospital in November 2017 with inappetence, progressive fatigue and neutropenia. The bone marrow biopsy was performed for evaluation of blood cytopenias. Cytogenetic analysis was performed at the time of diagnosis on bone marrow culture by trypsin-G-banding. FISH panel testing for ALL which includes eight different FISH probes (MYC rearrangement, CDKN2A, E2A rearrangement, MLL rearrangement, ETV6/RUNX1 translocation, BCR/ABL1 translocation, IGH rearrangement, and hyperdiploidy probes consisting of CHIC2, D10Z1, and D17Z1) (Cytocell, Cambridge, UK) was performed on the bone marrow cytogenetic pellet. The analysis was done in 200 interphase nuclei. The patient was diagnosed with B-ALL. There was no bone marrow metaphase spreads available for conventional cytogenetic analysis, however FISH examination revealed 3-6 copies of ETV6/RUNX1 fusion signals together with ETV6 deletion in 54% of cells. The classical ETV6/RUNX1 translocation was found in 15% of cells.

Conclusion: Report of rare numerical and structural genetic aberrations contributes to our understanding of the disease classification, prognosis and clinical management. However, a long-term follow-up is required to verify the possible prognostic effect of the ETV6/RUNX1 fusions amplification.

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P12.057A

Low level of TP53 mutation can be detected by NGS years before CLL clinical/laboratory diagnosis

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TP53 mutations are present in 10% of Chronic Lymphocytic Leukemia (CLL) patients at the time of diagnosis, and are associated with impaired survival and poor therapy response due to a selection of *TP53*-mutated chemotherapy-refractory clones. In order to assess the timing of somatic acquisition of clonal origin of *TP53* mutation, we performed a longitudinal deep next generation sequencing (NGS) in a patient with familial CLL. A clone and the subclone bearing deletion 11q and 13q switched from 80% and 45% at diagnosis to undetectable after chemotherapy (started 31 months after a therapy-free period), and *TP53*-mutated subclone with deletion on the 13q shifted from undetectable at the diagnosis to 80% after chemotherapy. A *TP53* pathogenic mutation (c.548C>G;p.Ser183*) was detected with a load of 93.8% in post-chemotherapy sample. Interestingly, the same mutation was present in a blood sample collected 16 months before clinical manifestation of disease with a mutational load of 15%. Risk stratification based on genetic prognostic markers is highly recommended at diagnosis to determine the most appropriate strategy for clinical management. Analysis of copy number variations, IGHV and *TP53* mutations can predict the aggressive clinical course of disease. Given the positive family history for CLL, pre-clinical stage NGS *TP53* analysis would have ranked the patient as a high-risk, low-responder individual, leading to opt for the last generation targeted therapies available. Given the low *TP53* mutational load, NGS test should be included in current clinical practice to ensure the best clinical management being the optimal technique to detect low mosaic mutations.

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P12.058B

The effects of the therapeutic agents Ponatinib and VS-5584 on Chronic Myeloid Leukemia leukemogenesis

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Chronic myeloid leukemia (CML) is characterized by cells with BCR-ABL. Leukemia stem cells (LSCs) are malignant derivatives of hematopoietic stem cells (HSCs) and cause resistance to chemotherapy. Ponatinib is ATP-competitive tyrosine kinase inhibitor (TKI) capable of inhibiting all BCR-ABL autophosphorylation, including T315I-mutant. VS-5584 is selective PI3K-mTOR dual-inhibitor, preferably killing cancer stem cells. We aim to investigate changes in apoptosis, cell cycle, oncogenic Akt pathway

phosphorylation, and transcription factor (TF) activity by targeting K562 and LSC with VS-5584 and/or ponatinib. Cytotoxic effects of VS-5584 and/or ponatinib on cell-lines were measured with WST-8. Combination indices were evaluated by isobologram analysis. Apoptotic effects were evaluated with AnnexinV and Caspase3 assays, effects on cell cycle were assessed using PI assay with flow-cytometry and by cyclinD1, p27 antibodies with western blot. Changes in TF activities and phosphorylated proteins were measured with dual-luciferase quantitation after transfection of constructs and PathScan Antibody-Array, respectively. It was determined that combinations were synergistic. In addition to ponatinib-induced apoptosis, VS-5584 was shown to cause marked G0/G1 arrest. Ponatinib in combination with VS-5584 was able to suppress Akt pathway through inhibiting the phosphorylation of several proteins including S6, S6K, BAD. In leukemic cells, suppression of SRE/Elk-1, AP-1, NFkB, CREB, myc/max, E2F/DP-1 and activation of C/EBP, FOXO, p53 TFs were more intense with VS-5584 treatment, compared to ponatinib treatment. HSCs were least affected. VS-5584 mediated suppression of BCR-ABL independent oncogenic pathways known to be active in CML, promises hope for the elimination of LSCs that can't be targeted with traditional TKI therapy.

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P12.059C

The expression of some HIF-1a regulated genes is connected with the differentiation of clear-cell renal cell carcinoma cells

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Clear-cell renal cell carcinoma (ccRCC) is the most common (70-80%) and aggressive among malignant neoplasms of the kidney. The mechanisms of its development are not fully known. It is important to obtain additional information on the molecular genetics mechanisms of ccRCC progression. In this study, the expression levels of 21 genes in the sample of 65 paired tumor/ normal renal tissue from patients with ccRCC were investigated by RT-qPCR. When analyzing the functional processes associated with the genes on Gene Ontology, three genes involved in the cell differentiation process (*ANGPTL4*, *BHLHE41*, *IGFBP3*) has been identified. These genes are activated by HIF-1α and the highest level of their expression in the initial stage of ccRCC was revealed. Significant reducing in expression levels of the genes at low degree of differentiation in relation to high degree was found for all three genes by U-test. The association study was carrying out using ROC analysis and the exact Fisher test. An association of the studied gene expression levels with the degree of tumor cell differentiation was significant by both tests: in the ROC analysis ($p = 0.0015$ - 0.035) and in Fisher test ($p = 0.008$ - 0.015). The application of the FDR correction to the multiplicity of comparisons retains the significance of association for all three genes. Thereby, a decrease of the expression level of the genes *ANGPTL4*, *BHLHE41*, *IGFBP3* in tumors of ccRCC is associated with a decrease in the degree of tumor cell differentiation, which becomes more malignant.

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P12.062B

The effect of solanine and dendrosomal nano solanine on expression of H19 lincRNA in CML cells

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Introduction: Chronic myeloid leukemia (CML) is a hematological disorder originated from a single oncoprotein encoded by BCR-ABL fused gene. Recently, the leukemia incidence has been grown meanwhile drug resistance and cancer recurrence have been occurred. Aberrant expression of some lncRNAs and consequently their association with tumorigenesis has been demonstrated in numerous cancers containing leukemia. Furthermore, recent studies introduced them as a noticeable target for therapy. The large intergenic non-coding RNA, H19, is abundantly expressed in CML cells and plays a meaningful role in leukemogenesis. Mentioned oncogenic lincRNA is tightly regulated by BCR-ABL transcript. Solanine is a natural glycoalkaloid with anti-proliferative effects has reported in diverse cancers. To investigate if solanine and DNS (dendrosomal nano solanine) have any considerable effect on H19 expression level, we designed further study.

Materials and Methods: BCR-ABL expressing cell line, K562, was cultured and treated with semi-cytotoxic concentration of solanine and DNS and incubated for 48 h. RNA extraction and cDNA synthesis was performed. The expression level of H19 was evaluated by real-time RT-PCR.

Results: ANOVA analysis revealed the down-expression of H19 in K562 cell line in only DNS treated group (P<

0.028). Down-expression was 1.8 times less in treated group in comparison to control group.

Conclusion: Suspending solanine in dendrosomal particles makes it more impressive in order to down-regulate H19 expression in k562 CML cells. Likewise DNS can be proposed as an effective alternative for targeted therapy if it brings about the same result in *in vivo* as well as *in vitro* model.

G. Asgari: None. **M. Sadeghizadeh:** None.

P12.063C

Germline cancer susceptibility in adolescents and young adults with colorectal cancer

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Colorectal cancer (CRC) at young age (≤25 years) is a rare condition. Two CRC syndromes causing such an early onset are familial adenomatous polyposis and constitutional mismatch-repair deficiency (CMMRD). However, the majority of CRCs ≤25 years are microsatellite-stable, are not associated with polyposis and show an increased mucinous histology compared to adult-onset CRC. These differences suggest that CRC ≤25 years represents a distinct clinical CRC entity.

We performed whole-exome sequencing (WES) on germline DNA from patients with CRC ≤25 years (n=40; for 15 cases trio-based sequencing was performed) and paired tumour samples (n=19) to unravel the underlying genetics of CRC at young age.

Six cases carried a pathogenic mutation in a known cancer predisposing gene: *BRCA2*, *NF1*, *POLD1*, *PALB2* (n=1 each), and *TP53* (n=2). One case carried a *de novo* mutation in the RAS-MAPK gene *SOS2*. *In vitro* expression of this mutant revealed an increase in ERK phosphorylation, suggesting a gain-of-function effect. The mutation in *POLD1* resulted in a frameshift and this patient presented with a hypermutated cancer resulting from an *in trans* somatic mutation affecting the active site of the exonuclease domain of *POLD1*. In addition, three cases presented with potentially pathogenic mutations in genes involved in the extracellular matrix.

Sequencing of tumour and paired germline samples and selective screening for *de novo* mutations provides a powerful strategy to investigate known and novel genes that predispose to early onset CRC. Our findings indicate that a genetic predisposition is frequent and heterogeneous in these patients.

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P12.064D

Evidence for *GALNT12* as a moderate penetrance gene for colorectal cancer

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Abstract

Characterizing moderate penetrance susceptibility genes is an emerging frontier in colorectal cancer (CRC) research. *GALNT12* is a strong candidate CRC-susceptibility gene given previous linkage and association studies, and inactivating somatic and germline alleles in CRC patients. We previously found rare segregating germline *GALNT12* variants in a clinic-based cohort (N=118) with predisposition for CRC. Here, we screened a new population-based cohort of incident CRC cases (N=479) for rare (MAF <1%) deleterious germline *GALNT12* variants. *GALNT12* screening revealed 8 rare variants. Two variants were previously described (p.D303N, p.R297W), and additionally, we found 6 other rare variants: five missense (p.H101Q, p.I142T, p. E239Q, p.T286M, p.V290F) and one putative splice-altering variant (c.732-8 G>T). Sequencing of population-matched controls (N=400) revealed an over-representation of these variants in CRC cases compared to healthy controls (P=0.04). We then functionally characterized the impact of these substitutions on GALNT12 enzyme activity using *in vitro*-derived peptide substrates. Three of the newly identified *GALNT12* missense variants (p.H101Q, p.I142T, p.V290F) demonstrated a marked loss (>2-fold reduction)

of enzymatic activity compared to wild-type (P≤0.05), while p.E239Q exhibited a ~2-fold reduction in activity (P=0.077). These findings provide strong, independent evidence for the association of *GALNT12* defects with CRC-susceptibility; underscoring implications for glycosylation pathway defects in CRC.

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P12.065A

Colorectal cancer bacteria microenvironment in different tumor genetic contexts

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Introduction: Cancer is a heterogeneous and complex set of multifactorial diseases that have in common an (epi) genetic origin. Colorectal cancers (CRC) with microsatellite instability (MSI) are a subset of hypermutated tumors generated by DNA mismatch repair deficiency. MSI tumors have specific pathological and clinical characteristics. There is a growing interest in knowing the putative role of the microbiota in the colorectal oncogenesis. We aimed to explore potential differences in microbiota in CRC patients with and without MSI.

Materials and Methods: Ninety-six DNAs from frozen tissues (tumor and normal mucosa) of 48 CRC patients (24 MSI and 24 microsatellite-stable-tumors (MSS)) were used to performed metagenomic 16S analysis (V3 and V4 region). Sequencing was conducted using a paired-end 2×300 bp cycle run on Illumina MiSeq. RDP Classifier was used for taxonomical assignment. Groups of data were compared using a paired and unpaired *t* test for normal vs tumor and MSI vs MSS, respectively.

Results: Globally, lower bacteria diversity (Shannon and Simpson indexes) was observed in tumor vs normal tissues. Diversity of MSI tumors vs their normal counterparts showed significant differences at genus level. Similarly, diversity of MSS tumors vs their normals were also lower at family and genus levels. Taxa differences among MSI vs MSS tumors were in families *Veilloneacea* ($p = 0.028$) and *Verrucomicrobiae* and genus *Akkermansia* ($p = 0.04$).

Conclusions: Differences on the colon microbiota composition associated to MSI/MSS tumor molecular phenotype were observed. Further studies are needed for a better understanding of these findings.

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P12.066B

Identification of mismatch repair-deficient colorectal cancers using a molecular inversion probe based sequencing assay of short mononucleotide repeats

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Introduction: UK's NICE guidelines recommend mismatch repair (MMR) deficiency testing of colorectal cancers (CRCs) to identify Lynch syndrome, a hereditary predisposition for CRC. Uptake of MMR deficiency testing has been poor due to the unsuitability of current assays - immunohistochemistry and fragment analysis - to high throughput testing, including manual workflows and results interpretation. We aimed to develop a next generation sequencing-based assay of short mononucleotide repeats to assess microsatellite instability (MSI), a biomarker of MMR deficiency, using single molecule-molecular inversion probe (smMIP) technology, with a view to improving the clinical uptake of MMR deficiency testing. Methods: 24 short mononucleotide repeats, together with clinically-actionable markers in *BRAF* and *KRAS*, were amplified in multiplex using smMIPs. Amplicons were sequenced using the Illumina MiSeq platform, aligned to reference genome hg19 using BWA, and analysed using custom R scripts. An MSI classifier was trained on short mononucleotide repeat sequences from 98 CRCs collected in pathology laboratories in Edinburgh and Spain, and validated in 98 CRCs collected at Newcastle.

Results: The MSI classifier showed 100% sensitivity and specificity, relative to fragment analysis, using either a 6 or a 24 marker panel. Frequencies of *BRAF* and *KRAS* mutations concurred with previous observations. Cost estimate for sample analysis using the proposed assay is £4.28/sample in contrast to £11.65/sample with fragment analysis.

Conclusions: Our novel MSI assay could streamline CRC molecular diagnostics by providing cheap and high throughput detection of MMR deficiency, acting as a companion diagnostic for immunotherapy and improving the identification of Lynch syndrome patients.

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P12.067C

Clinicopathological characteristics of patients with colorectal cancer in regards with microsatellite instability status in Ukrainian population: the pilot study

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Introduction: Identification of colorectal cancer (CRC) subtypes, especially microsatellite instability (MSI), is of great clinical importance because of its role in prognosis and predictioin of sensitivity to therapy. The lack of data on the prevalence of MSI in Ukrainian population, as well as its relationship with the clinical and morphological characteristics of patients makes actual to perform this study.

Materials and Methods: 177 patients with CRC who underwent MSI (26), or MMR (151 patients) testing were enrolled in the study. Demographic and clinical data were assessed in patients with MSI and MSS.

Results: The overall incidence of MSI among observed patients was 14.7%, while the incidence among men (25.35%) was significantly higher ($p = 0.0369$) than in women (10%). There were significant differences in age of patients with MSI (46.9 years) and MSS (57.1 years) (P=0,0020). Strong relation was found between MSI and tumor location in the proximal part of large intestine ($p < 0.0001$), regardless of sex. MSI was more often associated with medullary (100%) and mucinous (15.4%) histological types (P=0.0008), and these subtypes in 88.9% were detected in men. The strong relation of MSI with high grade was found - about 50% of patients with MSI had Grade 3 carcinomas independently of sex. However, patients with MSI demonstrated significantly lower association with distant metastasis (P<0.0001).

Conclusions: Thus, in Ukrainian population the frequency of MSI CRC is 14.7% and, as in other studies MSI was associated with male sex, younger age, right-sided location of tumor and high grade.

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P12.069A

Cytogenetic and molecular characterization of 33 complex variant Ph translocations diagnosed in patients with chronic myeloid and acute lymphoblastic leukemia

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Chronic myeloid leukemia (CML) is a myeloproliferative disease. The hallmark of the disease is the presence of a Philadelphia (Ph^1) chromosome produced by a reciprocal translocation t(9;22)(q34;q11). In 5-10% of the cases, the Ph^1 chromosome is generated by variant rearrangements, involving 9q34, 22q11, and one or more genomic regions. We report 33 cases of complex variant Ph^1 translocations diagnosed in 32 patients affected with CML and one patient affected with acute lymphoblastic leukemia (ALL). Cytogenetic and FISH studies were carried out in bone marrow samples. The third chromosome involved in the 33 complex variant translocations was the chromosome 1 ($n = 7$), 5 ($n = 5$), 12 ($n = 4$), 3, 11 ($n = 3$), 2,6,15 ($n = 2$), and 7,13, 17, 19, 20, 21 ($n = 1$). The total number of breakpoints were 34 (one of the translocations involved 4 chromosomes), and 26 were different. The q arm chromosome was the most frequently involved in the translocations (62%). The breakpoints were located in 81% of the cases in the G-light bands. Seven out of the 22 karyotypes showed one or 2 additional chromosomal abnormalities. FISH studies using the LSI BCR/ABL (VYSIS) probe were carried out in 24 out of the 33 cases allowing the detection of the fusion genes *BCR/ABL* on chromosome Ph^1 in all the cases. After FISH studies using LSI, CEP and WCP probes the karyotype was modified in 3 cases. Most of the breakpoints in our series are in the G-light bands. The combination of conventional cytogenetics and FISH studies allow us to identify these complex variant translocations.

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P12.070B

The use of circulating tumor DNA to study the genetic basis of treatment failure in rrDLBCL

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Introduction: Non-Hodgkin's lymphoma (NHL) is the 9th most common cancer in Europe, with 93,000 new cases and 38,000 deaths in 2012 alone. Diffuse Large B-Cell Lymphoma (DLBCL) is the most common subtype of NHL, and for DLBCL patients who fail primary treatment (rrDLBCL), prognosis is extremely poor, with a 5-year survival rate of around 10%. Thus, the genetic mechanism which lead to treatment failure must be identified to aid in the development of new molecular-based treatments.

Materials and methods: We have analyzed temporal samples obtained from 35 patients enrolled in two ongoing clinical trials. For each patient enrolled, a tumour biopsy sample is obtained prior to investigational treatment, as well as several blood samples as treatment progresses. Through a combination of whole exome and targeted sequencing of biopsy samples and circulating tumor DNA, the genetic landscape of rrDLBCL, and how it evolves in response to treatment, can be characterized.

Results: Prior to investigational treatment, we observed recurrent mutations in well-described lymphoma-associated genes (*KMT2D*, *EP300, EZH2, EP300*) as well as several genes associated with treatment failure (*CYP2A6, ABCA12, AHNAK2*) and metastasis (*NFBP1*) in other cancers. Following investigational therapy, mutations in several genes (*S1PR2* and *FOXO1*) were enriched overall, and subclonal populations containing these mutations expanded in several patients who failed treatment.

Conclusions: Mutations in lymphoma-associated genes may either directly contribute to salvage treatment failure, or act as a possible biomarker. Analysis of additional samples as they become available may identify other mutations.

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P12.071C

Comprehensive, integrated, and phased whole-genome analysis of the primary ENCODE cell line K562

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The chronic myelogenous leukemia cell line K562 is one of the most widely used in biomedical research. It is one of three tier-one cell lines of ENCODE, and one of the cell lines most commonly used for large-scale CRISPR/Cas9 gene-editing screens. Although the functional genomic and epigenomic characteristics of K562 are extensively studied, its genome sequence has never been comprehensively analyzed and higher-order structural features of its genome beyond its karyotype were only cursorily known. The high degree of aneuploidy in K562 renders traditional genome variant analysis methods challenging and partially ineffective. Correct and complete interpretation of the extensive functional genomics data from K562 requires an understanding of the cell line's genome sequence and genome structure. We performed deep whole-genome sequencing, mate-pair sequencing and linked-read sequencing to identify a wide spectrum of genome characteristics in K562: copy numbers of chromosomal segments, SNVs and Indels (both corrected for copy-number), phased haplotype blocks, structural variants (SVs) including complex genomic rearrangements, and novel mobile element insertions. A large number of SVs were phased, sequence assembled and experimentally validated. Several chromosomes show striking loss of heterozygosity. We re-analyzed K562 RNA-Seq and whole-genome bisulfite sequencing data for allele-specific expression and phased DNA methylation. We show examples where deeper insights into genomic regulatory complexity could be gained by taking knowledge of genomic structural contexts into account. Furthermore, we used the haplotype information to produce an allele-specific CRISPR targeting map. This comprehensive whole-genome analysis serves as a resource for future studies that utilize K562.

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P12.073A

Whole transcriptome sequencing > Kazakhstani patients with esophageal squamous cell carcinoma

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Introduction: Esophageal cancer is the eighth most common cancer in the world. The incidence rate in Kazakhstan is 10.1: 100 000. The aim of the project is to identify genetic basis of ESCC by performing whole transcriptome sequencing in Kazakhstani patients.

Materials and Methods: 54 patients with ESCC underwent surgery at Oncology center (Astana) between 2013 and 2016. Fresh frozen cancer tissue and its adjacent normal tissue specimen were obtained from each patient. Whole transcriptome sequencing was performed on Illumina platform using TruSeq RNA protocol. STAR software and DESeq package have been used for mapping and defining differentially expressed genes. MSigDB and KEGG databases were processed for analysis of signaling networks.

Results: According to the histological type of ESCC was prevalent moderately differentiated squamous cell carcinoma with infiltrative growth and without keratinization. In our patients the third stage of the disease (51.8%) was more often detected. Paired analysis of cancer and normal tissues identified 287 down-regulated and 192 up-regulated genes. Among up-regulated genes PPAR signaling pathway (p-value = 0.01), cytokine-cytokine receptor interaction (p-value = 0.05) and metabolism of lipids and lipoproteins (p-value = 0.03) have been identified. Whereas the most significant pathways among down-regulated genes are metabolism of xenobiotics by cytochrome P450 (p-value-= 1.31E-4), retinol metabolism P450 (p-value = 0.01), O-Glycan biosynthesis (p-value = 0.02).

Conclusion: Using whole transcriptome sequencing we could identify molecular pathways involved in esophageal tumorigenesis to understanding pathogenesis of ESCC and develop new diagnostic markers. This work was supported by grants of the Ministry of education and science #AP05134722 and # AP05135430

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P12.075C

Exosomal miRNA profiles in serum, plasma and platelets in healthy donors

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Introduction: Repository of human clinical specimen (biobanking) is the key to study genetic diseases. Among those, blood samples are the best resource of discovering serum/plasma biomarkers for liquid biopsies. Increasing evidence shows that exosomes, which can be found in all biofluids, play important roles in various kinds of diseases and are therefore good biomarkers. Besides, recent studies showed that platelets are able to intake and transport exosomes associated with several diseases, such as cancer. Hence, we aim to identify large-scale miRNA profiles of exosomes in serum, plasma, as well as those released by activated platelets.

Material and Methods: Serum, plasma, and activated platelets from 12 healthy donors are collected and used in this study. Exosomal miRNAs were isolated with ExoR-Neasy kit (Qiagen) and quantified using Human miRNA panel v3 on Nanostring nCounter system.

Results: A total of ~250 exosomal miRNAs are detected from all three resources: serum, plasma and activated platelets. Three groups of miRNAs are identified: higher expression in serum and plasma, higher expression in platelet, higher expression in serum and platelets. Overall, platelets have a distinct expression pattern comparing to serum and plasma. Interestingly, platelets show more overlapped genes with serum comparing to plasma, likely due to coagulation process in serum collection.

Conclusions: Our results suggest that serum possesses extra information of exosomal miRNAs from platelets, and therefore may be a better resource while applying repositories for studying diseases related to platelet functions.This study is supported by Research University Network on precision medicine in Thailand (MURA2017/747)

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P12.076D

Inherited *BRCA1* epimutation as a novel cause of breast and ovarian cancer

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Introduction: Pathogenic variants in *BRCA1* or *BRCA2* are identified in ~20% of families with multiple individuals with early-onset breast/ovarian cancer. Extensive searches for additional highly penetrant genes or alternative mutational mechanisms altering *BRCA1/2* have not explained the missing heritability. For the first time, we report transgenerational epigenetic silencing of *BRCA1* due to promoter hypermethylation in two families with breast/ovarian cancer.

Methods: *BRCA1* promoter methylation of ten CpG dinucleotides in breast/ovarian cancer families without germline *BRCA1/2* pathogenic variants was assessed by pyrosequencing and clonal bisulfite sequencing. RNA and DNA sequencing of *BRCA1* from lymphocytes was undertaken to establish allelic expression and the presence of germline variants.

Results: *BRCA1* promoter hypermethylation was identified in two of 49 families with multiple women affected with grade 3 breast/high grade serous ovarian cancer. Somawide *BRCA1* promoter hypermethylation was confirmed in blood, buccal mucosa and hair follicles. Methylation levels were ~50%, consistent with the silencing of one allele and confirmed by clonal bisulfite sequencing. RNA sequencing revealed allelic loss of *BRCA1* expression in both families and this segregated with a novel heterozygous variant c.-107A>T in the *BRCA1* 5'UTR.

Conclusion: Our results indicate a novel mechanism for familial breast/ovarian cancer, caused by epigenetic silencing of the *BRCA1* promoter, segregating with an *in cis* 5'UTR variant in two independent families. We propose that methylation analyses are indicated in all families affected by early onset breast/ovarian cancer without a *BRCA1/2* pathogenic variant.

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P12.077A
Reduced familial fertility in carriers of mutations in the BRCA1 and BRCA2 genes

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The frequency of founder mutations in *BRCA1* and *BRCA2* genes influences the genetic testing strategy for breast and/or ovarian cancer susceptibility in many populations. The aim of this study was assess clinical data associated with *BRCA1* and *BRCA*2 mutation-associated familial breast and/or ovarian cancer in the West Ukraine population. Previous genetic testing in a group of 125 women with familial breast and/or ovarian cancer identified 25 and 9 pathogenic mutations in *BRCA1* and *BRCA2* genes respectively. Clinical parameters were compared between groups of carriers and non-carriers of pathogenic mutations (34 and 91, respectively).Thirty three (97%) of the mutation carriers had at least one family members with the same gynecological cancer. In contrast, 24 (26%) of the non-carrier cases were the first breast and or ovarian cancer case in a family ($\chi 2=7{,}09$, $p < 0{,}01$; OR=11.82 [95% CI: 1.53-91,22]). In families with *BRCA1/2* mutation carriers there were numerous incidences of familial infertility and infertility of mutation carrier's children ($p < 0{,}05$). This study also

revealed a significantly higher incidence of medical abortion and spontaneous miscarriage (OR=7.84 [95% CI: 1.71-35.91]) that may be an independent risk factor for gynecological cancer in the group of non-carriers. In conclusion, the occurrence of the same gynecological cancer in relatives and reduced familial fertility may significantly increase the probability of carrying mutations in *BRCA1/2* genes in patients from West Ukraine.

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P12.078B

FFPE testing in deceased family members: implications for clinical management of patients seen in the genetics clinic

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Introduction: Archived pathology tissue can be a source of germline DNA for genomic analysis in families with no living affected individuals where the determination of mutation status can assist in the clinical management of living relatives.

Method: The Manchester Genomic Diagnostics Laboratory has developed and validated Next Generation Sequencing (NGS) based gene panels optimised for the detection of point and small insertion/deletion mutations in formalin fixed paraffin embedded (FFPE) tissue DNA. At present, the laboratory has analysed 80 FFPE samples from deceased breast and/or ovarian cancer index cases for variants in the BRCA1 and BRCA2 genes and 23 FFPE samples from deceased colorectal cancer index patients for variants in a panel of 13 genes implicated in inherited colorectal cancer.

Results: Successful analysis was achieved in 80% of cases and both pathogenic variants and variants of unknown clinical significance in the BRCA1 and BRCA2 genes, mismatch repair genes and the APC gene were identified. FFPE samples from as far back as 1988 have been successfully analysed. In total, of the 71 successful analyses with clinical data available, 55% of the results (17 pathogenic variants and 22 negative results) directly impacted the clinical management of relatives seen in the genetics clinic. This included changes to risk assessments, screening recommendations and the availability of predictive genetic testing.

Conclusion: Our data demonstrates how FFPE testing in deceased relatives is an accurate, informative and valuable tool in the clinical management of patients seen in the genetics clinic.

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P12.079C

Familial Intestinal Gastric Cancer: a polygenic or a monogenic disease?

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Introduction: Familial Intestinal Gastric Cancer (FIGC) remains genetically unexplained; despite has its autosomal dominant inheritance pattern. However, FIGC pedigrees often display generations without affected individuals, and late onset intestinal gastric cancer, indicating the presence of low or moderate risk alleles that may increase cancer susceptibility in FIGC.

We aimed at identifying the germline cause of FIGC and at characterizing the 2nd hits in potentially causative genes and other somatic events in FIGC tumours.

Materials and methods: Normal and tumour DNA from 52 FIGC probands were screened using a multiplex custom-panel of 67 cancer-associated genes. Somatic 2nd mutation and promoter methylation were searched by PCR-Sanger-sequencing.

Results: Twenty-four out of 52 FIGC families harboured germline variants. From these, 36 germline variants were found, affecting 17 genes, being *MSH6*, *SDHD*, *MAP3K6* and *ATM* the most frequently altered. Of notice, 42% of the families carried co-occurrence of germline variants. In fact, four families display germline and somatic variants with common features between different signaling pathways.

Further, two families displayed a 2nd mutation that potentially inactivates *MSH6* and *FAT4* genes at the somatic level. The somatic landscape revealed 115 variants, affecting 23 genes, found in 36 families. *TP53*, *MSH3*, *ARID1A* and *FAT4* were mutated more frequently.

Conclusion: In conclusion, this work pinpointed FIGC as a likely polygenic rather than a monogenic disease in 42% of FIGC families. Co-occurrence of low or moderate risk alleles may interact with family history and other non-genetic factors, increasing the risk of cancer.

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P12.080D
Differential expression of shelterin genes and telomere length variation in gallstone and gallbladder cancer patients of North Central India

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Introduction: Gallbladder cancer (GBC) has a very high prevalence in North Central India with an equally high mortality rate due to poor prognosis and lack of screening markers. Presence of gallstone/s is viewed as an important risk factor for GBC. A functional telomere, bound with shelterin protein complex, is central to maintaining genomic stability, failing which, may result in carcinogenesis. In this study we analyzed the role of telomere length variation and shelterin complex gene expression in GBC pathogenesis.

Materials and methods: Monochrome multiplex qPCR was performed to analyze telomere length and RT-qPCR was performed for expression analysis of shelterin genes (*TERF1*, *TERF2*, *POT1*, *TERF2IP* and *TINF2*) in 15 GBC and 10 gallstone patients. Statistical analysis was done using SigmaPlot v.11 software.

Results: Telomere length was found to be significantly reduced in tumor tissues, irrespective of the presence of gallstones. Increased telomere length was observed in gallstone (inflamed) tissues in comparison to both adjacent non-tumor and tumor tissues. Significant down-regulation of *TERF1*, *POT1* and *TINF2* genes was observed in inflamed tissues as compared to non-tumor and tumor tissues. Further, *POT1* was found significantly up-regulated in tumor tissues and specifically in tumor tissues with gall stones, compared to inflamed tissues.

Conclusion: Thus, our study suggests that telomere length remains unaffected in inflamed tissues, however, decreased expression of some shelterin genes may lead to improper telomere capping, paving the way for tumorigenesis. Also, *POT1* expression in gallstones patients could act as a diagnostic marker for GBC after further validations in future.

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P12.081A
Point mutation in exon 1B of APC in Czech families with GAPPS

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Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) is an autosomal-dominant cancer-predisposition syndrome with a significant risk of gastric adenocarcinoma. Li et al., 2016 described a few point mutations in the YY1 binding site of the APC gene 1B promoter associated with GAPPS syndrome. We performed Sanger Sequencing of APC promoter 1B (by Li et al., 2016) in 35 Czech families with previously unsolved cases of hereditary predisposition to familiar polyposis, gastric polyposis or gastric adenocarcinoma. The only pathogenic variant we detected in 1B promoter was APC NM_001127511: c.-191T>C mutation associated with GAPPS. Nine severely affected individuals from 7 unrelated families all of them carpeting of more than 100 fundic gland polyps and 4 of them already died of adenocarcinoma. We have performed predictive testing on asymptomatic family members and detected other 5 carriers of APC c.-191T>C mutation. Three of these carriers were diagnosed with more than 100 asymptomatic fundic gland polyps (born at 1988, 1983, 1968) and were recommended for prophylactic gastrectomy. Prophylactic gastrectomy was done in a carrier born 1988 and gastric adenocarcinoma was already present. No polyps were currently detected in one carrier woman born at 1987. Carrier mother (born at 1967) of the patient who died of generalized tubular adenocarcinoma at 28 years of age was ordered to gastroscopy. We

confirmed the presence of APC c.-191T>C mutation in several Czech high risk patients with gastric adenocarcinoma and/or proximal polyposis of the stomach. Supported by Czech Ministry of Health: MH CZ - DRO (MMCI, 00209805)

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P12.084D

Deregulation of TNF-a signaling pathway in gastric carcinogenesis mediated by miRNAs

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Introduction: Tumor necrosis factor (TNF)-α, a proinflammatory cytokine, can induce cell survival or apoptosis due to binding to TNFR1 or TNFR2 receptors. Both receptors may lead to cell survival, but only TNFR1 results in apoptosis, so deregulation in this pathway can promotes malignant progression. Thus, we investigated the mRNA and miRNAs expression involved in the TNF-α signaling pathway in gastric carcinogenesis and mRNA:miRNA interaction networks.

Materials and Methods: Relative quantification (RQ) of gene expression (*TNFA, TNFR1, TNFR2, TRADD, TRAF2, CFLIP, CASP8, CASP3, NFKB1*, *NFKB2)* and miRNAs (miR-19a, miR-34a, miR-103a, miR-130a, miR-181c) was assessed by the $2^{(-\Delta\Delta Ct)}$ method after RT-qPCR (TaqMan® assays) in 31 gastric cancer (GC) and 30 intestinal metaplasia (IM) tissue samples using normal mucosa pool as calibrator. ACTB/GAPDH and RNU6B/RNU48 normalized the mRNA and miRNA expression, respectively. Interation network were obtained by Cytoscape v3.1.1.

Results: IM showed downregulation of various mRNA as *TNFR1, TNFR2, NFKB1, NFKB2* and miR-19a, miR-103a and miR-130a. Contrary, GC showed upregulation most of the TNFA signaling pathway genes involved in cell survival as *TNFA, TNFR2, TRAF2*, *TRADD, CFLIP, NFKB2* and miR-34a, except *TNFR1* and *CASP3* that were downregulated. Correlation analyzes and interaction network showed various relationship between miRNAs:mRNA highlighting the miR-103a that target various TNF-α pathway genes.

Conclusion: TNF-α participates in the gastric carcinogenesis, favoring cell survival through the TNF-α/TNFR2/NF-κB pathway and blocking of *TNFR1*-mediated apoptosis, with predominance of anti-apoptotic mediators. miRNAs are deregulated and may influence the early stages of gastric carcinogenesis, suggesting a new altered pathway in gastric cancer. Financing: FAPESP, CNPq

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P12.085A

Mutations in different genes that regulate gastric chlorhydria explains from gastric neuroendocrine tumors to autoimmune thyrogastric pathologies

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Gastric neuroendocrine tumors (gNET) arise from enterochromaffin cell hyperplasia, which classically occurs in patients with autoimmune atrophic gastritis. Tumor progression entails gastric achlorhydria due to the destruction of parietal cells, which acidifies the stomach through the ATP4A proton pump. However, we have recently identified a mutation in the *ATP4A* gene that explained achlorhydria in a studied familial gNET. The knockin mouse model confirmed achlorhydria as the main effector of the tumor development. A second studied familial gNET case that segregated with two autoimmune diseases (Hashimoto hypothyroidism and rheumatoid arthritis) allowed us to describe a second mutated gene. The three individuals affected with gNET had two heterozygous mutations in the *ATP4A* and *PTH1R* genes (digenic model). The *PTH1R* gene, which directly regulates the gastric cells homeostasis and gastric chlorhydria, was also found involved in the regulation of Ca2+ by thyrotrophin (TSH) in the thyroid gland regulation pathway. We found that the cumulative effect of both mutations explained the gNET but also allowed us to link the gastric disease with both autoimmune pathologies (hypothyroidism and arthritis) with a common genetic origin. In order to deeper investigate this relationship, we have studied by WES five families with thyrogastric disease (chronic atrophic gastritis and hypothyroidism). In all families, we have found germinal deleterious mutations in new genes expressed in parietal cells that are involved in the regulation of gastric chlorhydria and Ca2+ homeostasis. This data has allowed us to compose a comprehensive genetic landscape of the biology of the gastric achlorhydria in thyrogastric disease.

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P12.086B

Spectrum of KIT gene mutations in Korean patients with gastrointestinal stromal tumors

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Background: The aim of this study is to dertermine the frequency and type of KIT gene mutation in Korean patients with gastrointestinal stromal tumors (GISTs).

Methods: Fourty four cases of GISTs were examined. DNA samples were extracted from hematoxylin/eosin-stained sections of representative paraffin-embedded blocks using a QIAamp DNA Mini Kit (QUIAGEN, Hilden, Germany). Bidirectional sequencing was performed using the BigDye Terminator v1.1 kit (Applied Biosystems, Foster city, CA, USA). Exons 9, 11, 13 and 17 of the KIT gene were amplified by PCR and sequenced.

Results: We detected mutations in 38 (86.4%) of the 44 patients with GISTs by direct sequencing. KIT exon 11 mutations were detected in 31 (70.5%) of 44 GISTs (19 deletions, 10 missense mutations, and 2 duplications); followed by KIT exon 9 mutations (5 duplications); and KIT exon 13 and exon 17 mutations in 1 each (2 missense mutations). Most of mutations consisted of the in-frame deletion from 3 to 51 bp in heterozygous fashion. Frame deletions and point mutations were most frequently observed at codons 550-580, but duplications were most concentrated at codons 502-503. Double mutation (exons 9 and 17) in metastatic GIST patient was detected in 1 (2.3%) of 44. KIT mutations were slightly more frequent in metastatic than in nonmetastatic GISTs (100% vs. 83.8%).

Conclusions: In most cases, the mutations were found at KIT exon 11, and in-frame deletions were the most common type.

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P12.087C

CD24 gene overexpression induces EGFR and NOTCH signaling pathways and EMT in Glioblastoma cells

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Gliomas are the most prevalent intracranial tumors and glioblastoma (GBM) is the most malign type among these tumors due to average short patient survival (12-15 months) and patients' limited response to the treatments. Therefore, efforts to better understand the biology of GBM require the development of effective therapies and prognostic markers that provide patient survival. One of these efforts is the discovery of the presence of cancer stem cells (CSC) which is correlated with the malignity of GBM. CD24, one of the most studied GBM stem cell markers, has been found to be prominently elevated in GBM tumors, indicating its involvement in tumor propogation. However, the molecular mechanisms underlying the tumor promotion in CD24+ GBM cells is still unclear. To understand one of these molecular mechanisms, we forced to overexpress CD24 in U87 GBM like cells in which CD133 and CD24 expression are low and then we performed CSC array by quantitative real-time PCR in CD24+ and CD24- U87 cells. We found that mRNAs of epidermal growth factor (EGF), integrin alpha-2 (ITGA2) or CD49b and mucin 1 (MUC1) were increased 2 folds, while DNA-binding protein inhibitor ID-1, jagged 1 (JAG1) and snail 1(SNAIL1) were increased 4 folds compared to those expressed in CD24- cells. These results suggest that CD24 induces activation of the EGFR, NOTCH signaling pathways and Epithelial Mesenchymal Transition. This research has been supported by The Scientific and Technological Research Council of Turkey (No:114S189).

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P12.088D

Gene expression with association of estrogen receptor status and tumor stage in breast cancer

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The choice of optimal therapy for breast cancer (BC) can depend on tumor features. In this connection, the level of 74 genes quantitative expression, selected bioinformationally as the most significant for the BC development, was performed in tumor in relation to the normal breast tissue. The sample of BC consists of infiltrative ductal BC on I-II stages. All patients of the sample were not subject to radiation, neither to chemotherapy. The gene differential expression on II stages in relation to stage I was revealed for eight genes: *BIRC5, CD151, mTOR, uPAR, ZEB1, FOXO1,*

KLF8, PDGFR ($p = 0.008$-0.04). The gene expression was studied in groups with estrogen-positive and estrogen-negative tumors. An increased expression of the *CSF1R* gene in the group with estrogen-positive BC ($OR = 17.3$, $p = 0.01$) was revealed. The *CSF1R* is a key pro-inflammatory cytokine that is involved in the recruitment and activation of tissue macrophages. Tumor-associated macrophages have been identified as regulators that enhance angiogenic, invasive and metastatic programming of neoplastic tissue. Our data suggest the increased recruitment of macrophages in estrogen-positive in comparison with estrogen-negative BC tumors.

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P12.089A
Gilbert syndrome and cancers

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Introduction: UDP-glucuronosyltransferase 1A isoform 1 is phase II xenobiotic biotransformation enzyme. Mutations in *UGT1A1* gene can cause insufficient glucuronidation of various endogenous (e.g. bilirubin) and exogenous substances (carcinogens and xenobiotics). Bilirubin is one of the strongest antioxidant in the body. Gilbert syndrome (GS) is one of the genetic disorders of bilirubin catabolism which is characterized by a moderate increase of total bilirubin. GS frequency is high (8-15%) in Caucasian, African and Afro-American populations due to the occurrence of TA dinucleotide insertion in *UGT1A1* gene promotor. The aim of this study was to determine whether people with GS with higher levels of total bilirubin are better protected against carcinomas than others.

Methods: The 3248 individuals analysed for TA insertion in *UGT1A1* promotor were divided into three groups: 1) group of patients with different types of carcinomas classified in subgroups, 2) control group and 3) group of individuals older than 60 years without personal and family history of cancer. Genotypic differences in these groups were statistically evaluated by chi square test.

Results: In all cancer subgroups and in group of individuals older than 60 years without carcinomas were found different frequencies of TA genotypes compared to the control group.

Conclusion: Herein we discuss the protective effect of bilirubin and UGT1A1 activity against the development of different types of cancers. The variances found in the genotypes of cancer subgroups are explained by their different etiology, as well as by genetic background of the organism, the influence of environment and lifestyle of the individuals.

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P12.090B
Carney-Stratakis syndrome in two patients with *SDHB/SDHC* genes mutations and Gastrointestinal Stromal Tumor (GIST)

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Gastrointestinal Stromal Tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. *KIT* and *PDGFRA* mutations account for 85-90% of GIST carcinogenesis. However, the remaining 10-15% of GISTs present loss of function mutations in genes encoding for the subunits of the succinate dehydrogenase (SDH) complex: SDHA, SDHB, SDHC and SDHD. The occurrence of SDH-deficient GISTs is restricted to stomach, and they typically occur in children and young adults representing a spectrum of clinical behavior from indolent to progressive. SDH-deficient GISTs can be due to either genetic or epigenetic changes. We report on two patients who were diagnosed with a gastric GIST at 14 years of age. Both tumors were c-kit-negative based on immunohistochemical staining. Next generation sequencing analysis on the tumor DNA samples revealed two homozygous mutations, a maternally inherited splicing variant and a de novo 1-bp frameshift deletion,

respectively in the *SDHB* and *SDHC* genes. The same mutations were confirmed in heterozygous state in the constitutional DNA from peripheral blood leukocytes. Germline mutations in *SDHB*, *SDHC*, and *SDHD* were seen in patients with the Carney-Stratakis syndrome (CSS), a very rare condition inherited in an autosomal dominant manner with incomplete penetrance, who are predisposed to developing paraganglioma and GIST. Here we report on two new cases of the CSS, with a gastric GIST, expanding the clinical and molecular spectrum of the disease.

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P12.091C

Impact of miRNA-138 in infiltrative and migratory ability and MMMI profiles of primary glioblastoma

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Introduction: Glioblastoma (GB) is the most common and aggressive entity from the primary tumors of the central nervous system shows a high infiltrative capacity. In previous studies, we have identified in primary GB, significant alterations in the expression of several miRNAs related to the different models of EGFR amplification and with MMMI changes and angiogenesis processes. In the present work we show the downregulation of miRNA-138 expression which, through its HIF-1 modulating action, acts as a transcription factor activated in hypoxia situations, with the ability of modulating behavior of neoplastic cells playing a role in tumorgenesis.

Material and Methods: We propose the development an in vitro model of study based on GB cell cultures, Allowing comparative analyses in cell lines and primary cultures of primary GB, with different levels of EGFR amplification: (i) in situations of miRNA-138 overexpression and silencing, (ii) in situations of EG FR and HIF mRNA inhibition (iii) in different situations of hypoxia.

Results: The miR-138 expression levels were stable under hypoxia conditions but decresed in normoxia conditions. miR-138 overexpression, an increase in the miR-138 values and a decrease of VEGF and HIF1a mRNA in both normal and hypoxic conditions were observed.

Conclusion: Our findings indicate that miR-138 is deregulated in GB and changed their expression levels within the different grades of GB. We reaffirm the possibility of miR-138 being able to act by modulating the changes in the MMMI, suggesting a potential role for these molecules in the pathogenesis of GB.FIS P114/01669 and PROMETEO II/2015/009

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P12.093A

Genetic alterations of gliomas

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Background: Understanding the genetic alterations that drive glioma formation and progression may help improve patient's prognosis and treatment. Here, we investigated gliomas samples from Northern Brazil to identify recurrent oncogenic copy number alterations (CNAs) and gene mutations.

Methods: In the present study, thirty-seven samples of gliomas were retrospectively analyzed in detail by molecular approaches, i.e. array-comparative genomic hybridization (aCGH), polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) followed by DNA sequencing and fluorescence in situ hybridization (FISH).

Results: CNAs were detected in 28/37 gliomas. The most frequent CNAs were loss of #9, #10, 13q, 22q and gain of #4, #7, #19 and #20. Of special interest among the detected CNAs are the following findings: gain of *BRAF* and amplification of *EGFR* genes (8.1% and 16.2%, respectively) and loss of *TP53*, *PTEN* and deletion of *CDKN2A* genes (8.1%, 16.2% and 24.3%, respectively). In addition, we found occurrence of mutations in hotspot regions of *TP53* in 12.2% of the gliomas, while no mutation was identified in *PTEN*.

Conclusion: High resolution molecular approaches combined in a cost-efficient way are an important tool to help in grading and understanding in future associations between genetic alteration in gliomas and prognosis. Higher rates of CNAs in highly malignant compared to low grade gliomas were identified. In addition, the *TP53* pathway is impaired, either by mutation or chromosomal loss, while allelic loss of PTEN could represent an alternative mechanism for its protein inactivation in gliomas development.

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P12.095C

Genetics of breast cancer in the Azores islands - BRCA genes

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Introduction: Breast cancer is the most prevalent cancer among women. Pathogenic variants in BRCA genes are the main cause of hereditary breast and ovarian cancer syndrome (HBOC); 7% of all breast cancers (BC) and 11-15% of ovarian cancers (OC) are associated with inherited predisposition, with a 40-80% lifetime risk of developing BC, and 11-50% of developing OC. The identification of carriers can significantly impact their and at-risk family members medical management.

Materials and Methods: The mutational profile and prevalence of BRCA variants were evaluated among 139 Azorean probands (cases) fulfilling the NCCN HBOC testing criteria. Variants were first scanned using HRM and the polymorphic fragments were Sanger sequenced. The clinical significance of the detected variants was assessed using ClinVar database. Variants not found in ClinVar were assessed in Ensembl, dbSNP, LOVD and BRCA Share databases.

Results: Seventy-four variants were detected in the samples analyzed: 33 in BRCA1 gene and 41 in BRCA2 gene. Three were pathogenic (BRCA1 c.2717delA and BRCA2 c.1138delA, c.2808_2811delAAAC), six were of uncertain significance, two had conflicting interpretations of pathogenicity and one, intronic with no significant splicing motif alteration, was not found in consulted databases (c.441+51T>C). The remaining 61 were classified as benign or likely benign.

Conclusion: The low percentage (2,2%) of pathogenic variants detected, in a group fulfilling NCCN HBOC testing criteria, suggests a strong contribution of genes other than BRCAs. To achieve a more efficient detection a broad panel of genes implicated should be applied.

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P12.096D

From the bench to the clinic. Identification of a CDH1 large rearrangement and management of the affected family

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Germline mutations in CDH1, encoding E-cadherin, account for around 30% of cases of Hereditary Diffuse Gastric Cancer (HDGC). Individuals carrying a pathogenic mutation in CDH1 have a high lifetime risk of developing diffuse gastric cancer, estimated to be 70% in men and 56% in woman. Female carriers also present an elevated risk to develop lobular breast cancer.

Identification of causal mutation allows predictive testing of family members and preventive care of carriers. Here we describe the identification of a large unpublished genomic rearrangement in CDH1 gene.

Molecular testing of the index patient by sequencing and MLPA methods revealed a deletion encompassing exons 10 and 11, leading to an expected frameshift and premature stop codon. RNA study was performed to exclude false positive result of the MLPA method and to confirm the pathogenicity of the mutation. The presence of a premature stop codon was confirmed and the mutation was considered as likely pathogenic. Familial screening was therefore proposed in this large family.

During this familial screening, the mutation was identified in several "old" healthy patients. This unexpected result leads us to the hypothesis that the mutation may show variable penetrance which complicate the follow-up and prophylactic recommendations to give to the family.

After multiple discussions with geneticists, CDH1 experts and gastro-enterologists, prophylactic gastrectomy was proposed to mutation carriers of the family. At this time, several carriers undergo surgery and pre-tumoral cells were identified in at least two of the carriers.

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P12.097A

A mutational chip to detect TP53, CDKN2A and FAT1 mutations in circulating cell free-DNA of head and neck squamous cell carcinoma patients:a pilot study

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is characterized by a high incidence of relapse, which is the common cause of death in HNSCC patients. The identification of biomarkers supporting the management of HNSCC disease is still an unmet need in clinical oncology.

Meterials and Methods: To this end, we designed a mutational chip for next generation sequencing (NGS) analysis to detect mutations in tumor tissues and matched plasma of HNSCC patients. The analysis of the HNSCC TCGA cohort revealed that TP53 (72%), CDKN2A (22%) and FAT1 (24%) are the most frequently mutated genes in HNSCCs. Notably TP53 mutations associate with poor outcome. A cohort of 250 fresh frozen tissues from HNSCC patients has been collected. Specifically, for each patient three biopsies representing non-tumoral (resection margin), peritumor (histologically tumor-free tissue at ≥1cm from the tumor) and tumor tissues were collected.

Results: This cohort has been challenged with a customized mutational chip for NGS analysis of mutations that includes the entire CDS of the TP53, CDKN2A and FAT1 genes. We found that the TP53 (743%) and CDKN2A (243%) mutation frequency in tumors of our cohort was similar to that of TGCA. While the frequency of FAT1 (38,6%) mutations was higher in our dataset. Many of the identified FAT1 mutations have not been annotated yet. Since we have also collected matched plasma at the surgery and during follow-up, our analysis is progressing toward the identification of mutations in ccf-DNA from patients.

Conclusions: This analysis is ongoing and the related data will be presented.

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P12.098B
Impact of cross-talk between laryngeal cancer cells and endothelial cells on cell migration and interactions

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Introduction: Endothelial cells constitute an important part of the tumor microenvironment (TME). However, the underlying mechanisms of their functions within the microenvironment of head and neck squamous cell carcinoma (HNSCC) remain poorly understood. Here we performed an *in vitro* non-contact co-cultivation system to analyze the influence of microenvironment in both tumor (laryngeal cancer cell line HEp-2) and healthy cells (endothelial cell line HUVEC cells).

Materials and Methods: We prepared conditioned medium (CM) from cell cultures for migration assay. CM has components secreted by cells such as specific cytokines, chemokines, growth factors. CM provides a co-culture microenvironments for the cells. We have used *in vitro* strach assay to examine cell migration and cell interactions. Photos were taken at intervals of 0, 3, 6, 9, 12 and 24 h. To assess the exact speed of cell migration, ImageJ software was used to measure the change in the cell-covered area over time, which is the characteristic parameter in migration assays.

Results: The wound healing assay showed that HEp-2 cells grown in CM from HUVEC have less capacity for migration and mobility compared with HEp-2 cells grown in the culture medium alone. HUVEC cells grown in CM from HEp-2 have less capacity for migration and mobility compared with HUVEC cells grown in the culture medium alone.

Conclusions: In vitro co-culture cell system for investigation of interaction between tumor cells and the tumor microenvironment was used. We believe that this study will contribute to our understanding of tumor microenvironment effect in head and neck carcinoma.

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P12.099C
Pathologies of helicases and premature ageing: study by derivation of induced pluripotent stem cells

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Helicases process the double-stranded DNA dissociation. They are involved in replication, DNA repair and

maintenance of telomeres. In human, 3 helicases display mutations responsible for clinical syndromes: WRN for the Werner syndrome, BLM for the Bloom syndrome and RECQL4 for the Rothmund-Thomson syndrome. All these diseases cause premature ageing and high risk of cancer. Molecular and cellular mechanisms involved in these diseases are not well defined. Particularly, little is known concerning the link between genomic instability and ageing.

During this project, we used blood samples and skin biopsies of affected patients to generate models by reprogramming cells to induced pluripotent stem cells (iPSCs). These cells have the advantage of self-renewing and theoretically could be differentiated in all cell types. At the same time, an iPSC senescence control was performed from cells of a Hutchinson-Gilford Progeria syndrome patient.

iPSCs were characterized for pluripotency. In the aim of recapitulate these pathologies *in vitro*, we identified sets of cellular and molecular phenotypes. We also engaged differentiation of iPSCs in cell pathways closed to the affected tissues *in vivo*. Finally, we studied the genomic stability of iPSCs and derived cells. We observed that Bloom cells are susceptible to frequent recombinations and are characterized by a genome instability through all studied cell types. Werner cells showed an instability of telomeres length. Finally, all premature ageing diseases displayed mitochondrial defects.

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P12.100D

Comprehensive molecular characterization of the transcriptome of HCC patients

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Hepatocellular carcinoma (HCC) is one of the deadliest cancer of the liver mainly due to late detection and limited interventional options. There is thus a need to better understand HCC to facilitate the development of better biomarkers and identification of potential targets for therapy. Next generation sequencing (NGS) of the transcriptome facilitates a more comprehensive expression profile of both host and viral genes including viral-host chimeric transcripts. In this study, deep sequencing of the transcriptome of HCC patients revealed that differentially expressed host transcripts are enriched in the cell-cycle, DNA damage response, cell-survival and apoptosis signalling and several of these were significantly associated with various clinical characteristics. An average of ~230 tumor-specific mutations were identified for each patients with most of these mutations being missense mutations within coding regions. Majority of the tumor-specific mutations were unique with only ~5% being found in more than a one patient. Genes harbouring somatic mutatio ns are primarily in cancer pathways affecting phosphatidyl inositol signalling and ubiquitin-mediated proteolysis. HBV-human chimeric transcripts were also observed in these HCC patients with majority of the HBV transcripts mapping to either the Pre-S or X gene. Interestingly fewer chimeric transcripts were observed in the tumor compared to the non-tumorous tissues. Majority of the HBV-human chimeric transcripts were primarily fusion of the HBx gene and introns or intergenic regions of human genes suggesting that favoured sites of integration within HBV remains at the C-terminal of the HBx gene. Acknowledgement: National Medical Research Council (NMRC) (CBRG14nov034 and NMRC/CBRG/0095/2015).

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P12.102B

Multi-gene testing of a high-risk group of Greek breast cancer patients reveals known and novel gene associations

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Introduction: Although *BRCA1* and *BRCA2* mutations dominate among breast cancer (BrCa) patients, additional genes are associated with BrCa susceptibility, which are nowadays analyzed simultaneously in multigene panels. The limitation in the implementation of such panels in the clinical setting is the uncertain clinical implications required in the case of mutations detected in genes other than *BRCA1/2*. Therefore, we sought to investigate the contribution and association of predisposing alleles in highly selected cohort of Greek BrCa patients.

Materials and methods: The sequences of 94 cancer genes were targeted by applying Trusight cancer panel in a high-risk group of 1382 BrCa Greek patients, diagnosed at very young age (<35years) and/or having strong family history (at least three breast, ovarian or pancreatic cancer cases). Case-control analysis was performed comparing the frequency of loss-of-function (LoF) mutations to Exome Aggregation Consortium controls.

Results: In total, 31.6% of patients tested carried LoF mutations in 26 genes; 6.7% carried LoF alleles in the post-BRCA genes, of which 4.5% involved in *CHEK2*, *ATM* and *PALB2* mutations. Case-control analysis showed established associations of *TP53*, *PALB2, ATM* and *CHEK2* truncating mutations (odds ratios 3.4-8.0), as well as novel associations of *RAD51C* and missense *CHEK2* mutations (odds ratios 6.19 & 3.79, respectively) to BrCa predisposition.

Conclusions: Among a high-risk group of Greek BrCa patients, where there are strong founder effects, the mutational spectrum is heterogeneous, giving rise to useful associations on known and candidate genes. Through such approaches, clinical actionability of genes, frequently included in panels, will be determined. <!--EndFragment-->

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P12.103C

BRF1, a new gene associated with hereditary colorectal cancer

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The identification of genes associated with hereditary colorectal cancer (CRC) facilitates the management of families and individuals carrying pathogenic mutations, having a direct impact in the processes of genetic testing and counseling. However, much of the genetic predisposition to CRC remains unexplained. We performed whole-exome sequencing in 3 CRC-affected relatives of an Amsterdam I CRC family. Variants located in 38 genes were shared by all affected relatives. A splice-site mutation in BRF1 (subunit of RNA polymerase III transcription initiation factor), stood up as potential causal mutation. BRF1 mutational screening was performed in 547 additional familial CRC cases using pooled DNA and targeted next generation sequencing. Ten novel or rare (population MAF<1%) BRF1 variants were identified in 11 independent CRC families. The deleterious nature of the identified BRF1 mutations were demonstrated for seven of them (1 detected in two families): BRF1 c.1459+2T>C and 6 missense variants, p.T12M, p.V75M, p.S81T, p.C140S, p.P405R and p.R572G, which led to the alteration of protein function and/or protein expression in functional studies carried out in yeast and/or human CRC cell lines. The frequency of mutations in familial CRC cases was significantly higher to the frequency observed in control population. Germline heterozygous mutations in BRF1 may contribute for at least 1.4% of unexplained familial colorectal cancer cases. If validated in independent series, BRF1 mutation carrier families could benefit in the future from a clinical management based on carrier status and personalized risk assessment.

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P12.104D

Screening for germline variations of cancer related genes in patients with the diagnosis of different cancers and hereditary cancer predisposition syndromes

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Introduction: Cancer genetics is a developing area of cancer researches. Next Generation Sequencing (NGS) is a powerfull technology, becoming an important tool offering opportunities to solve the complexity and multigenic nature of cancers and hereditary cancer predisposition syndromes (HCPS). In this study we aimed to summarise the results of genetic analysis performed in a population of patients with hereditary colorectal (n = 6), hereditary breast/ovarian cancers (n = 7), neurofibromatosis type I/II (n = 9), paraganglioma (n = 2), pheochromocytoma (n = 1), tuberosclerosis (n = 1) and malign mesothelioma (n = 1).

Materials and Methods: After genomic DNA isolation from peripheral blood, Trusight Rapid Capture Kit (Illumina Inc., San Diego, CA) has been used for the Sequencing reactions according to manufacturer's instructions. MiSeq Reporter (v2.5.1; Illumina Inc.) was used for fastq generation and Genomize Seq Platform was used for variant calling and filtering. ClinVar and HGMD databases were considered for known variant interpretation, Varsome was used for in silico analysis and ACMG 2015 guidelines were followed for the interpretation of novel variants.

Results: In total, 16 (9 novel) pathogenic and 3 likely pathogenic variations have been determined in 18 out of 28 patients (64.2%). Segregation analysis of the the variants classified as unknown significance is ongoing in the patients who have available family members.

Conclusion: We suggest that multi-gene panels with NGS is a powerfull tool for determining genetic background of cancers and cancer-related syndromes. Besides some disadvantages like the probability of false positivity, NGS is holding a great advantage in terms of time and cost effectiveness according to conventional methods.

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P12.105A

Detection of a variety of mutations in cancer predisposing genes in familial & early-onset colorectal cancer patients using targeted multigene panels

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Abstract

Introduction: Early onset and family history of cancer are the strongest predictors of hereditary colorectal cancer (CRC) risk. Mendelian CRC predisposition syndromes underlie about 5% of all cases. Despite this knowledge, the remaining CRC heritability is still unexplained and may be caused by rare or population-specific variants in known candidate genes or other cancer-related genes. Here we aim to identify known or novel colorectal cancer predisposing gene variants in early-onset and familial CRC cases.

Materials and Methods: Forty-eight CRC patients with family history or early age of onset were enrolled in this study. Genomic DNA was extracted from blood samples, library construction and targeted capture of 94 cancer genes were performed using the Illumina Trusight cancer panel followed by sequencing at approximately 80x coverage on an Illumina MiSeq instrument.

Results: In total, 65 variants in 30 genes were identified. Seventeen patients (35,4%) had a pathogenic mutation in one of the known CRC predisposing genes including: *MLH1* (3 cases), *MSH2* (4 cases, with two novel variants), *MSH6* (2 cases), *MUTYH* (3 cases, biallelic) and *APC* (4 cases, with one novel variant). In addition, rare and novel pathogenic variants were detected in 25 genes known to play a role in different cancer pathways.

Conclusions: Our findings demonstrate the power of using targeted sequencing of a broad panel of genes in clinical diagnosis of hereditary CRC and highlight the potential role of other cancer-related genes in the disease as

well as the potential pleiotropic effects in promoting germline predisposition to CRC.

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P12.106B

Specific issues in genetic counseling of hereditary leiomyomatosis and renal cell cancer (HLRCC)

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Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant inherited tumor syndrome predisposing to the development of multiple cutaneous piloleiomyomas, early onset uterine leiomyomas (myomatous uterus) and also to a clinically aggressive form of type 2 papillary renal cell cancer in approximately 15% of affected individuals. HLRCC is caused by heterozygous germline mutations in the *fumarate hydratase* (*FH*) gene. At present more than 300 families with HLRCC have been described.

We report three independent families that underwent genetic testing and counseling for HLRCC at our institute. The renal cell cancer occurred in two families, one family presented with cutaneous and uterine leiomyomas only. Each family harbored a different mutation in the *FH* gene. Two mutations (out of frame deletion c.912_918delTTTTGTC and splice site mutation c.905-1G>A) were definitely pathogenic. One missense mutation (c.977G>A; p.Gly326Glu) was novel, very likely pathogenic and a further segregation analysis has been recommended.

Based on the examples of our families we discuss various counseling issues and more general aspects of this rare tumor syndrome. These include clinical criteria, predictive genetic testing of children, renal cancer surveillance, possible underdiagnosis of HLRCC in the general population and prospects of immunohistochemical screening of HLRCC-associated tumors.

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P12.107C

HLRCC-associated renal cell carcinoma and renal cysts in paediatric patients: two case reports and review of the literature

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Introduction: Hereditary leiomyomatosis and renal cell cancer (HLRCC) is a rare, autosomal dominant syndrome caused by pathogenic germline mutations in the fumarate hydratase (*FH*) gene. It is characterized by cutaneous and uterine leiomyomas and an increased risk of developing renal cell carcinoma. Currently, there is no consensus on what age to start surveillance for renal tumours in *FH* mutation carriers, and whether the presence of renal cysts is an indication for more intensive surveillance or resection.

Materials & Methods: We report two paediatric patients with HLRCC-associated renal cell carcinoma and/or renal cysts and reviewed the available literature for HLRCC-associated renal tumours occurring in patients <20 years.

Results: Localized HLRCC-associated papillary type II renal cell carcinoma was successfully resected in a 15-year-old female patient with a family history of leiomyomas. In an unrelated 14-year-old female with a confirmed *FH* mutation (c.1210G>T(p.(Glu404*)), complex renal cysts were detected upon first screening and carefully monitored by regular MRI's. Nine previously described cases of HLRCC-associated renal cell carcinoma were identified in patients aged 10-18 years: four papillary type II, one collecting duct tumour, four unspecified. Five were metastatic at diagnosis. Atypical cells have been reported in the lining of resected renal cysts in adult *FH* mutation carriers, suggesting a potential preneoplastic lesion.

Conclusions: HLRCC-associated renal cell carcinoma can occur at early age and uniform recommendations are warranted for genetic testing and surveillance of young *FH* mutation carriers. The presence of complex renal cysts seems to require careful monitoring, and resection when solid components appear.

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P12.108D

Cell-free, long non-coding RNA as novel biomarker in the diagnosis of ovarian cancer

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Background: Ovarian cancer is the fifth leading cause of cancer-associated mortality among women. A reliable, non-invasive diagnostic method may contribute to the early detection of this malignancy. The long non-coding RNAs (lncRNAs) have a significant role in the oncogenesis, metastasis and chemoresistance. The upregulation of Hox transcript antisense intergenic RNA (HOTAIR) was determined in the development of ovarian cancer cells, however, the cell-free HOTAIR expression was not detected from the plasma until now.

Materials and Methods: Twenty previously untreated ovarian cancer patients (60.60 ± 10.84y; FIGO stages III or IV) and 20 healthy controls (56.70 ± 14.51y) were involved in the study. EDTA blood was drawn; RNA was isolated; cDNA was synthesised and the HOTAIR lncRNA expression were determined by using ExiLERATE LNA™ qPCR, for mRNA and long non-coding RNA (Exiqon, USA). ACTB was used as reference gene. Student t-test was applied for the statistical calculations.

Results: Higher expression of HOTAIR was detected in the samples of patients with ovarian cancer (1.89 ± 2.39) than in healthy controls (1.39 ± 1.48) but the difference was no significant ($p = 0.432$).

Conclusion: We determined the concentration of the cell free HOTAIR lncRNA from the plasma of ovarian cancer patients and healthy controls. There was no significant difference in the expression of the analysed lncRNA; but this is the first study to analyse the cell-free HOTAIR expression among Hungarian ovarian cancer patients. We would like to extend our study on higher number of cases as this lncRNA seem to be novel biomarker for the diagnosis of ovarian cancer.

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P12.109A

Clinical and biological manifestation of RNF168 deficiency in two Polish siblings

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Introduction: Germline mutations in the RING finger protein gene *RNF168* have been identified in a combined immunodeficiency disorder called RIDDLE syndrome. Only two patients have hitherto been described with somewhat different phenotypes.

Methods: We performed exome sequencing on two Polish siblings who presented with immunoglobulin deficiency, telangiectasia, cellular radiosensitivity and increased alpha-fetoprotein levels. We investigated the levels of RNF168, ATM and ATM target phosphorylation using Western blotting, and we monitored the DNA damage response after 6 Gy irradiation using immunocytochemical analyses of 53BP1 and pSer139-H2AX.

Results: Exome sequencing identified homozygosity for a novel frameshift mutation, c.295delG, in the *RNF168* gene for both siblings. The younger sibling with a more pronounced neurological and morphological phenotype also carried an *ATM* gene mutation in the heterozygous state. Immunoblot analyses showed absence of RNF168 protein, whereas ATM levels and function were proficient in LCLs from both patients. 53BP1 recruitment to DNA double-strand breaks after irradiation was undetectable in cell lines from either of the two patients. pSer139-H2AX foci accumulated normally but they disappeared with significant delay, indicating a severe defect in DNA double-strand break repair.

Conclusions: These findings together with the two previously identified cases define immunoglobulin deficiency, cellular radiosensitivity and increased AFP levels as the hallmarks of RNF168 deficiency. The failure of 53BP1 recruitment after irradiation may serve as a useful marker to screen for further patients.

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P12.110B

miR-15a, miR-15b and checkpoint gene expression among bladder cancer cell lines as a model of tumour heterogeneity

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Introduction: The complex immunological synapse between T lymphocytes and cancer cells contains checkpoint proteins, which modulate the signal transmitted to T lymphocytes. The role of miRNAs from the miR-15/16 family in bladder cancer remains unknown, although some research suggests its downregulation. Only a small fraction of bladder cancer patients respond to immunotherapy. The present study is aimed at the evaluation of the correlation between the expression of these miRNAs and the expression of checkpoint mRNAs in bladder cancer.

Materials & Methods: Three cell lines: MGHU4, 5637 and SW1710 (low to high level of genome instability), were used as a model of bladder cancer. The expression was analysed using RT-PCR, statistical and graphical analyses (Spearman Rho correlation, Benjamini-Hochberg FDR) were performed using R tools and XLstat.

Results: The expression of most checkpoint genes is positively correlated to the expression of any other analysed checkpoint gene. The expression level of miRNAs is negatively correlated with several checkpoint genes, showing high level of heterogeneity between cell lines. There is a negative correlation between genome instability within the cell line and expression of mir-15b.

Conclusions: Many checkpoint genes are co-expressed together and the expression of *mir-15a* and *miR-15b* is negatively correlated with them. This suggests that these miRNA negatively regulate the expression of checkpoint genes at the synapse, at the post-transcriptional level. The expression level of checkpoint genes together with their interconnections might be crucial for successful immunotherapy. Revealing such novel interconnections may aid in the development of new diagnostic tools, outcome predictors or immunotherapeutic drugs or combinations thereof.

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P12.112D
A rare case of colorectal cancer with coexistance of two different KRAS mutations

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Driving mutations in the RAS genes represent a negative predictor for personalized treatment decision in patients with colorectal cancer (CRC). Although mutations in these genes are mutually exclusive, few cases (2%) have been described with coexistence of two different mutations in the same gene. Such data are not avalaible for Romanian patients. The present study reports a case of coexistence of two different somatic mutations in exon 2 (codons 12 and 13) of KRAS gene, in a 68 years old Caucasian woman suffering from mixt adenoma (tubulo-papillary).Genomic DNA was isolated from FFPE primary tumor, from the same selected area, with 58% tumor cells content, and tested for KRAS exon 2 mutations by PCR-RFLP method followed by capillary sequencing.

From 2013 we have performed the screening of KRAS exon 2 for 550 patients suffering from advanced CRC. To our knowledge, this is the first Romanian case reported with coexistence of two different somatic mutations, in an early stage of colorectal carcinoma. One mutation was detected and confirmed in codon 12 as c.34G>T (p.Gly12Cys). The other mutation was detected in codon 13 but could not be confirmed by capillary sequencing, being probably under LOD, which is 20% in our laboratory. Based on epidemiological data, KRAS mutations in adenoma stage represents a very rare event compared with adenocarcinoma stage. Our results suggest the need for KRAS exon 2 screening even in early stages of colorectal cancer, contributing in this way to effective selection of CRC patients prior to personalising treatment decision.

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P12.114B
Pathogenic alterations in advanced HPV-negative cell squamous laryngeal carcinoma revealed by Next Generation Sequencing

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Introduction: There is still no enough data on the genetic alterations in laryngeal squamous cell cancer (LSCC), detected by NGS. The aim of our study was to explore the somatic status of patients, diagnosed with advanced laryngeal carcinoma by NGS techniques and its clinical impact.

Methods: In the current study were included 38 HPV-negative patients with advanced LSCC. The isolated DNA was used for Next Generation Sequencing of 48 tumour-associated genes (TSACP) by MiSeq (Illumina). The data was analyzed by VarSeq Software (v.1.4.6).

Results: The results showed 60 pathogenic mutations in 23 tumour associated genes. The most commonly mutated gene was TP53, with 28 (46.67%) variants with pathogenic prediction. We found 7 new pathogenic variants in TP53 gene in 7 patients. The second most mutated genes were PIK3CA and FBWX7 with 4 pathogenic variants each. We found all together 27 (45%) non published variants with pathogenic predictions in CDH1, CDKN2A, ERBB4, FBXW7, NOTCH1, PIK3CA, SMARCB1, TP53, FGFR2, FLT3, GNAQ, GNAS, KRD, NRAS, SMAD4, STK11, VHL genes. One benign and known pathogenic variants, relevant to cancer treatment, were found in KRAS, PIK3CA and TP53.

Conclusion: In conclusion, molecular profiling of laryngeal cancer with NGS could reveal the genetic architecture of LSCC and offer opportunities for prediction of response to existing target therapies and finding new therapeutic targets, which is a step forward in individualized medicine.

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P12.115C

Investigation of thymoquinone effect on laryngeal cancer cells with *KRAS* mutant

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Introduction: Larynx cancer constitutes about 2% of all cancers and 25% of head and neck cancers. *KRAS* oncogene is a member of the RAS gene family. When *KRAS* is mutated, the gene functions as "open" as a result. *KRAS* mutation has been shown to be effective in the development of head and neck cancer. Phyto-therapy practices are subject to rapid cancer research in the world. Since active ingredients of many drugs are plant original, the interest to plants increases. Many plants used in the early conventional treatments have become the subject of numerous important molecular research studies. Thymoquinone is one of the active ingredients of *Nigella sativa* known also as the black cumin. Many of the biological effects of these seeds have been shown that they are through the effect of thymoquinone. Introducing the apoptotic activity of thymoquinone on laryngeal cancer provides basis to investigate the therapeutic efficacy of this ingredient.

Materials and Methods: The apoptotic effect of thymoquinone on the laryngeal cancer cell line was determined by MTT and Sulphurodamine B cytotoxicity tests. Its effect on the mutant *KRAS* gene transfected cells was observed through MTT and intracellular ATP analysis.

Results: Thymoquinone has cytotoxic effect on the laryngeal cancer cells and cell death was decreased in the cells carrying mutation in the coding region of *KRAS* gene. However, cell death increase was observed in cells having mutation in the coding and non-coding region of *KRAS* gene.

Conclusions: It was observed that thymoquinone interacts with KRAS oncogene.

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P12.116D

Expression of *LINC01330*,long intergenic non-protein coding RNA 1330, in serous ovarian carcinoma

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Introduction: Among common cancers in women, ovarian cancer has the lowest incidence; however, mortality rates are more than other gynecological cancers due to the lack of specific symptoms during early stages and absence of diagnostic markers. Comparative genomic hybridization (CGH) demonstrated high frequency of 3q25-26 copy number gains (CNGs) in ovarian carcinomas. Serous papillary carcinoma as a common type of epithelial ovarian cancer shows singular copy number variations (CNV) of this region. This chromosomal region contains many protein coding genes and some of them have an oncogenic role, including *PIK3CA*, *Sox2*, and *HTERC*. *LINC01330 (LRRC77P)* which is a Pseudogene, located in this controversial region and led us to make more extensive studies of its expression alteration in serous ovarian cancer. According to following study, *LINC01330* has shown a remarkable change.

Materials and Methods: In this study, expression of *LINC01330* was evaluated in fresh frozen tissues. Total RNA was isolated from the tissues and cDNA synthesis was performed. Semi-quantitative RT-PCR technique was used and the validity of PCR products was determined by Sanger sequencing. The expression level of *LINC01330* was evaluated by real-time PCR. Result: Our finding revealed

the down-expression of *LINC01330* in tumor tissues (P<0.01). Down-expression was 2.5 times less in tumor group in comparison to control group.

Conclusion: Lack of specific markers in ovarian cancer is the vital barrier against early detection and finding molecular markers with specific expression alteration will help us for better diagnosis. Therefore LINC01330 can be proposed as an effective and sensitive biomarker.

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P12.117A
Development of a noninvasive method for assessing circulating tumour DNA in patients with solid tumours

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Introduction: It is estimated that more than 14 million people are diagnosed with cancer each year. In 2012, over 583,000 deaths were caused by lung, colorectal, breast, prostate, and ovarian cancer accounting for over 45% of all cancer-related deaths in Europe. Current diagnostic methods rely on molecular and histopathology results derived from invasive tissue biopsy, which has several inherent drawbacks; it is time-intensive, costly and not always accessible. Furthermore, it is associated with risks to the patient while localised sampling misses cancer heterogeneity. However, circulating tumour DNA (ctDNA) in plasma may provide a novel non-invasive means for early cancer detection, diagnosis, and monitoring not otherwise possible with conventional testing.

Materials and Methods: We developed an assay based on a proprietary form of targeted in-solution hybridisation for the detection of somatic mutations in the patient's plasma. A total of 523 driver and clinically actionable mutations across 49 genes are targeted. Reference material was used for the proof of concept study and plasma and matched-tissue samples from non-metastatic breast cancer patients were used for pre-clinical validation.

Results: Our proof of concept study exhibited LOD between 0.1-1% MAF. Preliminary experiments with clinical samples from patients with breast cancer showed concordance of the mutational profile of the tissue and matched plasma.

Conclusions: We aim to use circulating cell free DNA as a biomarker coupled with our targeted NGS-based approach to develop a liquid biopsy assay that provides safe and highly accurate non-invasive testing for solid cancers.

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P12.118B
Monitoring the EGFR T790M mutation in liquid biopsy in patients with lung cancer

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Molecular EGFR analysis from liquid biopsy is increasingly being used to monitor response to tyrosine kinase inhibitor (TKIs) therapy in lung cancer patients.

Our ongoing study aimed to monitor the EGFR T790M mutation status in patients with non-small cell lung cancer (NSCLC), which is the most common mechanism of resistance to TKIs.

The molecular EGFR analysis was performed by real-time PCR on plasma DNA collected from 34 patients (aged 33-81 years) with advanced NSCLC, who received EGFR-TKIs therapy based on EGFR activating mutation detected at diagnosis (by the same method, applied on DNA extracted from FFPE tissues between 2012-2017). Blood samples were collected from patients at the time of disease progression (3-58 months, median of 13.5 months) after the initial EGFR analysis (exon 19 deletion (19del) in 27 patients, L858R mutation in 7 patients and S768I mutation in 1 patient). Additionally, the initial EGFR activating mutation was also detected in liquid biopsy in 28 (82.35%) patients (21 with 19del, 6 with L858R mutation and 1 with S768I mutation).

Our results suggested that the EGFR T790M mutation occurs more frequently in NSCLC patients with EGFR 19del than in patients with L858R, possibly due to the fact that EGFR 19del mutants of NSCLC have a distinct biological phenotype that would favor the acquisition of T790M mutation, as other literature studies have hypothesized.

Our results are potentially important for clinical decision-making in NSCLC patients with EGFR mutation. Study on larger group of NSCLC patients is ongoing to establish a certain statistical significance.

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Detection of BRAF *V600E* mutation by droplet digital PCR in plasma of patients with malignant melanoma

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Introduction: Melanoma is the most aggresive form of skin cancer. About 50% of melanomas have BRAF V600Emutation. This mutation is an attractive therapeutic target. Detection of BRAF V600E mutation is a potential prognostic factor in malignant melanoma. Here, we studied BRAF V600E mutations in circulating cell-free DNA in plasma („liquid biopsy“) by droplet digital PCR (ddPCR).

Materials and Methods: We analyzed 100 patients with malignant melanoma. Circulating DNA from plasma was isolated by using QIAamp DSP Virus Kit (Qiagen, Hilden, Germany). DNA quantification was performed in a Qubit 2.0 fluorometer. ddPCR was performed with QX200 system (BIO-RAD®, Hercules, USA). All samples were tested in duplicate.

Results: The Limit of Detection was determined by the method of Tzonev. The LOD was found to be 5 events per well. The BRAF V600E mutation was detected in 37/100 samples.

Conclusion: ddPCR is a highly sensitive method and could be use for routine laboratory detection of BRAF V600E mutation as well as follow-up to treatment response in patients with malignant melanomas. This work was supported by the "Biomedical Center Martin (BioMed Martin)" ITMS code 26220220187 project which is co-financed from EU sources and by the Slovak Research and Development Agency under the contracts no.APVV-16-0066 and no. APVV-14-0273.

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Identification of tumor suppressor microRNAs by integrative miRNA and mRNA sequencing of matched tumor normal pairs in lung adenocarcinoma

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Roles of microRNAs (miRNAs) have not yet been explored systematically at the genome scale in lung cancer biology despite their important regulatory functions. Here, we report an integrative analysis of microRNA and mRNA sequencing data for the matched tumor and normal samples from 110 Korean female patients with non-small-cell lung adenocarcinoma. We produced miRNA-Seq and RNA-Seq data for 49 patients and RNA-Seq data only for additional 61 patients. Differential expression analysis with stringent criteria yielded 44 miRNAs and 2,322 genes. Integrative gene set analysis of differentially expressed miRNAs and genes using miRNA-target information yielded several processes related to cell cycle regulation that were targeted by tumor suppressor miRNAs. We performed the colony formation assay in A549 and NCI-H460 cell lines to test the tumor suppressive activity of down-regulated miRNAs in cancer and identified 7 novel tumor suppressor miRNAs (miR-144-5p, miR-218-1-3p, miR-223-3p, miR-27a-5p, miR-30a-3p, miR-30c-2-3p, miR-338-5p). Two miRNAs of miR-30a-3p and miR-30c-2-3p showed differential survival characteristics in the TCGA LUAD patient cohort, suggesting their prognostic value. Our study not only provides a massive dataset of miRNA and mRNA sequencing from the matched tumor-normal samples but also reports several novel tumor suppressor miRNAs that could be further developed into prognostic biomarkers or RNA therapeutic targets.

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Germline mutations in young non-smoking women with lung adenocarcinoma

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Objectives: Although the primary cause of lung cancer is smoking, a considerable proportion of all lung cancers occur in never smokers. Gender influences the risk and characteristics of lung cancer and women are over-represented among never smokers with the disease. Young age at onset and lack of established environmental risk factors suggest genetic predisposition. In this study, we used whole population-based sampling of the youngest patients in Finland to discover candidate predisposition variants for lung adenocarcinoma in never-smoking women.

Materials and Methods: We employed archival tissue material from 21 never-smoker women who had been diagnosed with lung adenocarcinoma before the age of 45, and exome sequenced their germline DNA.

Results and Conclusion: Potentially pathogenic variants were found in eight Cancer Gene Census germline genes: *BRCA1*, *BRCA2*, *ERCC4*, *EXT1*, *HNF1A*, *PTCH1*, *SMARCB1* and *TP53*. A nonsense variant in *TP53*, and missense variants in *BRCA1* and *BRCA2*, are likely to have contributed to the early onset lung cancer in the respective patients. This supports the notion that lung adenocarcinoma can be a component of certain cancer predisposition syndromes. Fifteen genes displayed potentially pathogenic mutations in at least two patients: *ABCC10*, *ATP7B*, *CACNA1S*, *CFTR*, *CLIP4*, *COL6A1*, *COL6A6*, *GCN1*, *GJB6*, *RYR1*, *SCN7A*, *SEC24A*, *SP100*, *TTN* and *USH2A*. Four patients showed a mutation in *COL6A1*, three in *CLIP4* and two in the rest of the genes. Some of these candidate genes may explain a subset of female lung adenocarcinoma predisposition.

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P12.123C

***CHRNA3*, *CHRNA5* and *CHRNB4* Polymorphisms Mapping and Correlation with Lung Cancer Susceptibility in Thai Population**

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Introduction: Several risk factors of lung cancer were known, for example; air pollutions, smoking and genetic variations. However, the information about environmental-gene interaction associated with lung cancer in Thai population is limited. Recent genome wide association studies in Europeans identified a few regions related to lung cancer susceptibility including 15q25.1 region, containing nicotinic acetylcholine receptor genes. This study aimed to investigate the association between genetic variations, exposure to environmental risk factor and lung cancer occurred in Thailand, and for further study of some clinical significance, the onset of disease evaluation in heavy smokers, and the prognosis in lung cancer.

Materials and Methods: Participants were recruited from healthy volunteers, heavy smokers and lung cancer patients who attended hospitals in Bangkok from 2012-2016. All samples were examined serum heavy metal levels and genotyped for *CHRNA3*, *CHRNA5*, and *CHRNB4* polymorphisms such as rs1051730, rs6495309, rs16969968, and rs17486278 using TaqMan Genotyping Assay Systems. The association between each minor allele frequencies and lung cancer and heavy metal levels were assessed by logistic regression analysis. Age, sex, and smoking consumption were included as covariates. Haplotype frequencies were calculated and LD blocks were drawn by Haploview software.

Results and Conclusions: *CHRNA5* polymorphism showed association with both nicotinic consumption and risk for lung cancer development as well in Thai population. Heavy metal levels was different between groups of patients, however the association between nicotinic receptor polymorphisms and heavy metal metabolism should be explored with larger studies. The structure of LD block will be shown in the poster.

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Analysis of somatic mutations in lung cancer with targeted next generation sequencing

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Introduction: Small cell lung cancer(SCLC), adenocarcinoma(ADC) and squamous cell lung carcinoma(SCC) could be discriminated at molecular level by specific "driver mutations" in many tumour-associated genes. Targeted next generation sequencing(NGS) allows simultaneous analysis of many genes and more complete characterization of the somatic mutations in tumours.

Materials and Methods: In the current study DNA isolated from fresh-frozen tumour tissues of 12 patients with ADC, 12 with SCC and 13 SCLC were included. Hotspot somatic mutations in a panel of 48 tumour-associated genes (TSACP) were analysed by NGS platform MiSeq(Illumina).

Results: The performed analysis showed 23 pathogenic variants in ADC samples.The most frequently mutated gene was *TP53* with 12 pathogenic mutations(52,17%).Eight activating mutations(34,78%) were found in *KRAS*. Other pathogenic variants were observed in *APC*,*RB1,BRAF* with frequency of 4,35% each.In SCC samples 21 pathogenic variants were detected:12 variants in *TP53*(57,14%),5 activating mutations in *KRAS*(23.82%) and mutations in the genes *GNAS*,*PIK3CA,SMO,STK11* with frequency of 4,76% each.In SCLC 23 pathogenic variants were observed. The most frequently mutated gene was *TP53* with 12 pathogenic variants(52,17%),followed by *Rb1*(30,76%) and *BRAF*, *EGFR,FGFR2,NOTCH1,PIC3CA,PTPN1, SMARCB1* with one mutation each-(4,35%). Around 50% of all analysed samples harboured more than 1 pathogenic mutation.

Conclusion: Our results suggest that the NGS analysis of lung cancer using a panel of tumour-associated genes is able to detect somatic mutations that are currently used as predictive biomarkers for targeted therapies.In addition it reveals the spectra of specific "driver" mutations and provides opportunities for the discovery of new therapeutic targets and personalized treatment. **Acknowledgements:** This work was supported by Grants 508/21.01.2016/ Contract №24/2016/MU-Sofia;DUNK01/2/2009/NSF.

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Whole exome sequencing to discover lung tumor predisposition in women with previous breast cancer

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Introduction: About 10% of women with breast cancer (BC) develop a second unrelated tumor including lung cancer (LC). The risk increases in radiotherapy-treated patients and in smokers. Since the current radiotherapy does not fully justify this risk, we hypothesize that genetic predisposition could enhance LC development and performed whole exome sequencing to unravel it.

Materials and Methods: At Ospedale Policlinico San Martino, Genova, 28 women with LC after BC (Study Population, SP) and 32 women with BC only (Control Population, CP) were enrolled. Genomic DNA was extracted from FFPE tumor and normal samples. Libraries were prepared with Agilent SureSelect All Exon and sequenced on Illumina HiSeq 2500. Variant calling was performed with FreeBayes. Somatic signatures were calculated on all nucleotidic substitutions and burden tests were computed using WSS and C-α statistics.

Results: Two mutational signatures were extracted: S1, similar to COSMIC-S30 (unknown etiology), included all SP-BC and 16/28 LC; S2, enriched in 12/28 LC, positively correlated with smoking and matched with COSMIC-S4, linked to tobacco use. These signatures may reflect two distinct mutagenic processes underlying LC development: smoking could have played a major role in S2-LC subgroup while genetic predisposition could enhance LC development in S1-LC patients. Therefore, we performed a gene-based burden test over rare germline variants in S1-LC versus CP and we identified 249 candidate genes (FDR<0.05).

Conclusions: Our results show two mutational signatures underlying LC development. Germline data validation step is ongoing to confirm them in an independent cohort.

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P12.126B

Birth order affects risk of multiple lymphoid cancers and allergies in lymphoid cancer families

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Introduction: Lymphoid cancers are a heterogeneous group of neoplasms that arise from immune cells. Familial clustering of lymphomas support a genetic contribution to cancer predisposition; however, infectious diseases and the immune system have also been implicated. The hygiene hypothesis proposes that a lower infectious burden during early life inhibits the immune system from maturing optimally. Consequently, such individuals are more susceptible to atopic disorders, including allergies and autoimmune disease, and some lymphoid cancers.

Methods: We characterized atopic conditions in lymphoid affected sibships of 182 families with a history of lymphoid cancers. Early life data was collected from telephone interviews and questionnaires from multiple family members.

Results: 314 sibships had 405 lymphoid affected and 927 unaffected siblings. An inverse relationship between birth order and risk of cancer was observed for all lymphoid cancers collectively ($p<0.0001$), and separately for multiple myeloma ($p=0.0015$), non-Hodgkin lymphoma ($p<-0.0001$) and individual B-cell subtypes, including chronic lymphocytic leukemia ($p=0.0124$), follicular ($p=0.0217$) and marginal zone lymphoma ($p=0.0169$). We also observed an inverse relationship between birth order and risk of allergies ($p=0.0284$), for both environmental allergies ($p=0.0465$) and multiple allergies ($p=0.0114$) in lymphoid affected individuals.

Conclusions: Childhood exposures to infectious diseases may play a role in immune dysregulation and subsequent risk of multiple types of lymphoid cancers, and allergies. The familial nature of the cancers implies shared genetic and/or environmental factors. There is a need for further evaluation of lifestyle factors that may protect against lymphoid cancers even in the familial context.

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P12.127C
Breast cancer in Irish patients with Lynch syndrome

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Pathogenic variants in mismatch repair genes *MLH1, MSH2, MSH6, PMS2* and more rarely *EPCAM* cause Lynch syndrome (LS), conferring variable risks of endometrial, colorectal, upper gastro-intestinal, urinary and biliary tract cancers, depending on gene and type of variant. Evidence for associations between LS and Breast Cancer is conflicting[1], with a recent report suggesting the risk may be limited to pathogenic variants in *MSH6* or *PMS2*[2]. We report a female patient with multi-focal breast cancer associated with a germline variant in *MSH2*. A 37-year old female, presented with multifocal, ER-positive, invasive ductal breast cancer. Her father (deceased) developed colon cancer at 37 years of age. Analysis of his tumour identified microsatellite instability, and immunohistochemical (IHC) analysis identified absent staining for MSH2 and MSH6 proteins. IHC of our patient's breast tumour confirmed mismatch repair deficiency, and subsequent germline testing identified a likely pathogenic splice-site variant (c.2210 +1G>C) in *MSH2*. We have objectively demonstrated a clear causal relationship between an MSH2 variant and breast cancer in one individual. Considering the frequency of breast cancer, it is unsurprising that a proportion of families with LS include members affected by the condition. We plan to analyse pedigrees of other Irish families with LS to further elucidate the frequency of breast cancer in this cohort. The penetrance of such variants for breast cancer is unclear, and additional surveillance for this cancer in this population may not be cost-effective. 1. Win et al., Breast Cancer Research, 2013, 2. Roberts et al., Genetics in Medicine, 2018

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P12.128D
Comprehensive genotypic and phenotypic characterization of heterozygous versus homozygous MMR gene mutation carriers in an Indian cohort

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Introduction: MMR gene mutations when heterozygous cause adult onset autosomal dominant Lynch Syndrome (LS), in homozygous state they cause autosomal recessive

Constitutional MisMatch Repair Deficiency (CMMRD) syndrome characterized by a variety of childhood cancers and café-au-lait spots. The MMR gene mutation phenotype, which is strikingly different depending on its zygosity, has not been described in any Asian population.

Method: Based on personal and family history of cancer, phenotyping and tumour IHC, 50 LS and 10 CMMRD families were identified. One or more MMR genes (MLH1, MSH2, MSH6 and PMS2) were sequenced followed by large genomic rearrangements (LGR) analysis. To avoid pseudogene amplification, long range PCR was done for PMS2. If no mutation / LGR was identified in these 4 genes, extended gene panel test was done which included MLH3.

Result: In the 50 families with suspected LS, heterozygous pathogenic mutations (including 6 LGRs) were detected in 46 (92%) families (31-MLH1, 14-MSH2 and 1-MSH6) and a homozygous MLH3 mutation in a 30 yr old female with endometrial cancer and family history of colon cancer. In the 10 CMMRD suspected families, biallelic germline PMS2 mutations were identified in 4 families (novel homozygous frameshift mutations in 3, compound heterozygous mutation in 1).

Conclusion: While phenotypic manifestations of heterozygous MMR mutations are in concordance with previous reports, we expand the CMMRD spectrum with the first report of an adult onset endometrial caner in a biallelic MLH3 carrier. This largest single centre study of CMMRD suspected families identifies a high prevalence of biallelic PMS2 mutations.

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P12.129A

DNA methylation changes and somatic mutations as early events in Lynch syndrome-associated colorectal cancer

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Lynch syndrome (LS) is caused by germline mutations in DNA mismatch repair (MMR) genes resulting in increased cancer risk, including colorectal cancer (CRC). Early events of multistep tumorigenesis accelerated by germline and somatic changes remain obscure. This study defines DNA methylation changes at different stages of LS-associated CRC, and somatic mutations in adenomas.

Colorectal biopsies including normal mucosae, adenomas and carcinomas were prospectively collected from 104 LS patients, supplemented with retrospective tumor specimens. DNA methylation was analysed using methylation-specific multiplex ligation-dependent probe amplification test (MS-MLPA) for selected tumor suppressor genes (TSGs), for CpG island methylator phenotype (CIMP)-associated markers, and LINE-1. Immunohistochemistry was used to detect MMR protein expression in tumors. As an ongoing study, mutation statuses of adenomas are investigated using the Ion AmpliSeq^{TM} Colon and Lung Cancer Panel.

The MMR protein expression decreases along with dysplasia but occurs relatively late in tumor progression, suggesting other somatic events to drive tumorigenesis. Methylation of TSGs and frequency of CIMP increases and LINE-1 methylation decreases in tumors along with dysplasia. In MMR-proficient (MMR-P) and MMR-deficient (MMR-D) adenomas, LINE-1 methylation decreases along the loss of MMR protein expression, and interestingly, higher methylation of *SFRP1* is observed in MMR-P adenomas. Furthermore, certain CRC-associated somatic mutations (e.g. *KRAS*) appear prevalent in MMR-P adenomas.

Concluding, similarly to sporadic CRC, early appearance of epigenetic changes and somatic mutations are important in LS-associated tumorigenesis.

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P12.130B

High cancer risk in two cases of constitutional *MLH1* epimutation

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Introduction: Constitutional epigenetic inactivation of the *MLH1* gene represents a minor cause of Lynch syndrome

(LS), but the identification of this condition is relevant for cancer risk assessment and clinical management of the patients. We describe two cases of constitutional *MLH1* hypermethylation without any apparent linked variants in the *MLH1* promoter.

Case descriptions: A 30-year-old woman with colorectal cancers (CRC) and a man with multiple cancers (urothelial cancer at age 48, two CRCs at ages 59 and 69, sebaceous adenomas at age 61) referred to genetic counselling as suspected for LS. The family history in both cases was negative for Amsterdam criteria.

All cancers but urothelial one of both patients showed high microsatellite instability (MSI-H) and loss of immunohistochemical MLH1/PMS2 protein expressions. No germline *MLH1* and *PMS2* pathogenic variants were diagnosed using both NGS and MLPA technical approaches.

Epigenetic somatic analyses were performed by bisulfite pyrosequencing and MS-MLPA on all cancers and dense biallelic *MLH1* methylation pattern was observed in all MSI-H neoplasms. Hemy-allelic methylation was detected in peripheral blood samples of both patients and no constitutional *MLH1* methylation was observed in the parents of one patient.

Conclusions: The LS flow-chart including somatic genetic and epigenetic analyses is crucial for diagnosis of this subset of Lynch-Like patients. The identification of constitutional primary epimutation has an important clinical impact for carriers showing a high risk of developing LS-related cancers. On the contrary, their relatives, as it is not an inherited condition, have a general population's cancer risk.

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P12.131C

Contribution of *MUTYH* variants to male breast cancer risk: results from a multicenter study in Italy

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Introduction: Male breast cancer (MBC) is a rare disease, whose etiology appears to be associated with genetic factors. Inherited mutations in *BRCA1/2* genes account for about 10-15% of all cases. Biallelic germ-line mutations in the DNA repair gene *MUTYH* cause MUTYH-associated polyposis (MAP) syndrome, whereas monoallelic mutations are reported in families with both colorectal and breast cancer. We aimed to test if *MUTYH* germ-line mutations may contribute to MBC susceptibility.

Materials and Methods: We screened the entire coding region of *MUTYH* in 560 MBC cases by a multi-gene panel analysis. The presence of all variants detected was also analyzed in 1540 male controls using a TaqMan approach.

Results: Biallelic *MUTYH* pathogenic variants (c.536A>G and c.721C>T) were identified in one case with phenotypic manifestation of adenomatous polyposis. Monoallelic pathogenic variants were identified in 14 (2.5%) MBC cases, in particular: c.536A>G in 7 cases, c.1187G>A in 5 cases, c.734G>A and c.933+3A>C in 1 case, respectively. Increased MBC risk in association with c.536A>G emerged (OR = 4.60; 95% CI: 1.19–17.81; p = 0.027).

Conclusion: Our results suggest that *MUTYH* pathogenic variants may have a role in MBC, in particular *MUTYH* c.536A>G variant may be a low/moderate penetrance risk allele for MBC. Moreover, our results suggest that MBC may be part of the tumor spectrum associated with MAP syndrome, with implications in the clinical management of the patients and their relatives.

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P12.132D

GERMLINE INVESTIGATION IN DNA REPAIR GENES OF MALE BREAST CANCER BY NEXT-GENERATION SEQUENCING

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Male breast cancer (MBC) is a rare disease, its incidence is $1/10^5$ year and it represents less than 1% of all breast cancers. MBC tends to occur between 60-70 years old and to express oestrogen and progesterone receptors frequently. Subsequently, luminal subtype is the most common phenotype with occasionally epidermal growth factor receptor 2 amplification. The rarity of MBC has precluded the large clinical trials, thus genetic predisposition remains not well understood. In order to better define genetic risk factors in men, a germline investigation in MBC cases was performed through the screening of 24 genes involved in breast cancer predisposition, genome stability maintenance and DNA repair mechanisms by Next Generation Sequencing. Clinical pathological data and family history of 81 MBC cases were collected. The average age of onset was 61.3 years and 35 men showed breast cancer family history. Our results let us to attribute a genetic cause to breast cancer in 23% of cases. In total, 19 patients carried a pathogenic mutation in 4 genes: *BRCA2, BRIP1, MUTYH* and *PMS2*. As expected, a positive family history is a strong predictor of germline *BRCA2* mutations. Moreover, 14 variants of unknown clinical significance (VUS) in 9 genes (*BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *ERCC1*, *NBN*, *PALB2*, *PMS1*) were predicted as potentially pathogenic by *in-silico* analysis leading to 40% the mutation detection rate. Understanding the potential pathogenicity of VUS represents an extremely urgent question for the implementation of breast cancer risk management in MBC cases and their own families.

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P12.133A

Intratumoral heterogeneity of melanoma as revealed by whole-exome sequencing

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Tumors continuously evolve to maintain their growth; secondary mutations increase the proliferation, resulting in high heterogeneity of tumor cells. To study the clonal architecture of melanoma samples the whole-exome sequencing of three patient's primary tumors and metachronous metastases was performed using NextSeq 500 system. Somatic mutations were annotated and clustered based on their frequency. The clusters were ordered regarding to their cellular prevalence to reconstruct the tumor clonal evolution. One patient carried BRAF V600E mutation in primary tumor and in a metastasis after targeted therapy with vemurafenib. Additional mutated genes in metastatic cells were PMS2 (16%) and CYP21A2 (19%). The second patient had BRAF V600E (27%), PMS2 c.706-4delT (23%), CTNNB1 c.95A>G (15%) both in tumor and metastasis, but TERT c.835G>A was presented in 64% of primary tumor cells and only in 15% of metastasis. The third case was presented by tumor (M0) and three metastases differed by localization and time from initial diagnosis (M1, M2, M3). BRAF V600E was identified in all samples except M1. However, in M1 the mutated MICAL1 gene was presented almost in all cells comparing with 4% in M0. The MICAL1 gene is known to control cell growth and survival of V600E melanoma cells. The last in time M3 carried additional somatic mutations in ANK3, DCDC1, STAB2, FLT1, ZNF638, ACVR1C, SNAP91 genes. Thus, reconstruction of tumor clonal evolution is important for understanding further tumor progression and mechanisms of resistance to anticancer therapy.The work was supported by the Russian Science Foundation (grant # 14−35-00107).

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P12.134B

New familial melanoma susceptibility locus at 11q identified by genome-wide linkage analysis in Spanish melanoma-prone families

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Melanoma etiology is complex and involves environmental, phenotypic and genetic factors. Approximately 10% of melanoma cases occur in a familial context, but the main genetic factors for familial melanoma remain unknown in more than 75% of families. CDKN2A is mutated in around 20% of melanoma-prone families, while other high-risk melanoma susceptibility genes explain less than 3% of families studied to date.We performed the first genome-wide linkage analysis in CDKN2A-negative Spanish melanoma-prone families to identify novel melanoma susceptibility loci. We included 68 individuals from 2, 3 and 6 families with 2, 3 and at least 4 melanoma cases. Subjects were genotyped on either the HumanOmni2.5 (Illumina) array versions v1.0 (81% of subjects) or v1.1 (19% of subjects).We detected a locus with significant linkage evidence at 11q14.1-q14.3, with a maximum het-TLOD of 3.449 (rs12285365:A>G), using evidence from multiple pedigrees. The genes contained by the subregion with the strongest linkage evidence were: DLG2, PRSS23, FZD4 and TMEM135. We also detected several regions with suggestive linkage evidence (TLOD>1.9) (1q, 6p, 7p, 11q, 12p, 13q) including the region previously detected in melanoma-prone families from Sweden at 3q29. The family specific analysis revealed three loci with suggestive linkage evidence for family #1: 1q31.1-q32.1 (max. TLOD 2.447), 6p24.3-p22.3 (max. TLOD 2.409) and 11q13.3-q21 (max. TLOD 2.654). Future next generation sequencing studies of these regions may allow the identification of new melanoma susceptibility genetic factors.Acknowledgments: Instituto de Salud Carlos III (15/00716,15/00483), AGAUR 2017 SGR1134, "CERCA Programme / Generalitat de Catalunya", NCI of the US NIH (CA83115).

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P12.136D
Novel, thyroglobulin-embedded microRNA gene deregulated in papillary thyroid carcinoma

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MicroRNAs are short, non coding RNAs. Aberrant expression of miRs is observed in numerous cancers, leading to tumor development, growth and progression. With use of the next-generation sequencing we discovered, a putative non-coding RNA (miR-TG) encoded within the sequence of thyroglobulin (*TG*). *In silico* and *in vitro* analysis confirmed that putative miR-TG is microRNA. Next, the downregulation of miR-TG and *TG* in papillary thyroid carcinoma (PTC) was confirmed. The expression of the novel miR-TG and *TG* was decreased to 44% ($p = 0.04$) and to 48% ($p = 0.001$) in PTC compared with unaffected tissue. Using *in silico* tools we identified *MAP4K4* as target gene for miR-TG. To confirm direct interaction between the miR-TG and 3'UTR *MAP4K4* the Dual Luciferase System was used. Analysis of transcriptome by RNA-seq proved that overexpression of miR-TG in PTC-derived cell line downregulates several genes, including *MAP4K4* (fold change 0.82; $p = 0.036$). We confirmed this result by SQ-PCR. The level of *MAP4K4* was lowered to 0.71 ($p = 0.004$). PTC-derived cell line transfected with miR-TG reveals increased proliferation.We propose that miR-TG plays a fine-tunning role in proper function of thyroid gland and its downregulation potentially can lead to activation of MAPK kinases, underlying initiation and progression of thyroid carcinogenesis. This work was supported: Preludium DEC-2012/07/N/NZ3/02033 (to M.K.); Opus DEC-2013/11/B/NZ3/00193 (to K.J.); LIDER/017/299/L-5/13/NCBR/2014) (to A.W.)

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P12.137A
Association of microRNA expression with metastases in gastric cancer

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The association of microRNA expression with metastasis in gastric cancer (GC) was studied. Two approaches were used: a hybridization of separate from tissue microRNA on NanoString chip containing more than 800 microRNAs, and expression investigation by quantitative real time PCR of bioinformationally selected a set of microRNAs important for tumor development. The sixty paired tumor / normal samples from patients with early-stage GC and metastatic

GC were analyzed. The decreased expression of mir34a, mir146a and mir335 was associated with distant metastases among gastric cancer patients. These microRNAs act as tumor suppressors and a decline of their expression level can affect the tumor progression. Expression of miRNA among patients with the same tumor size (T4) with varying degrees of regional lymph node involvement in metastasis (from 0 to 15) was investigated using the NanoString CSO / Human v3 miRNA chip, which allows selection without preliminary amplification of the studied microRNAs. It was found that mir146a expression was significantly reduced (by more than 10 times) in tumors of patients with a high degree of regional lymph node involvement (10-15) in comparison with tumors without metastases. In summary, reduced expression level of mir34a, mir146a and mir335 is associated with distant metastases. The decreased expression level of mir146a is also associated with early metastasis of the GC tumor that can suggest its possible involvement in starting of metastasis process. A participation of microRNA in distant metastasis may not be obligatory connected with early-stage metastasis.

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P12.138B
Frequency and molecular fingerprints of microsatellite instability across multiple cancer types

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Introduction: Microsatellite instability (MSI) is a hypermutable phenotype caused by defective DNA mismatch repair system. Although MSI has been well described in colorectal adenocarcinoma, less is known about the prevalence and distinct molecular features of MSI among other types of cancer.

Materials and Methods: We examined MSI across 14 cancer types (n= 596) by employing the Promega MSI Analysis System which used 5 mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) to identify MSI in a tumour and normal tissue DNA. Within a subset of samples with detected MSI we assessed mutation burden and distinct molecular signatures associated with this tumour phenotype.

Results: We identified MSI in 7% of all examined malignancies (present in 7 tumour types; table 1). MSI phenotype was frequently observed in EC (20.2%) and STAD (16.7%). Most notably, MSI was detected in ALL (5%), LIHC (4.9%), KIRC (3.5%) and MESO (2.6%) in which MSI has not yet been well described. MSI-high GBM had the highest mutational load among all MSI-positive samples (mean, 16,432 ± standard deviation, 16,301; $p < 0.001$) suggesting that such tumours will better respond to checkpoint blockade immunotherapy. We also discovered that MSI-H ALL and EC harbour significant number of somatic fremeshift mutations in DNA repair genes.

Conclusion: Our findings reveal the landscape of MSI across different malignancies and underscore the need for further studies of this tumour phenotype.

Prevalence of MSI across 14 cancer types

Cancer Type	№ of samples	MSI-H	MSI-L	% MSI
1)Endometrial carcinoma (EC)	89	14	4	20.22
2)Stomach adenocarcinoma (STAD)	78	9	4	16.67
3)Glioblastoma multiforme (GBM)	57	4	1	8.77
4)Pediatric acute lymphoblastic leukaemia (ALL)	39	2	0	5.13
5)Liver hepatocellular carcinoma (LIHC)	41	1	1	4.88
6)Kidney renal clear cell carcinoma (KIRC)	29	1	0	3.45
7)Mesothelioma (MESO)	38	1	0	2.63
8)Breast invasive carcinoma (BRCA)	54	0	0	0.00
9)Ovarian serous cystadenocarcinoma (OV)	45	0	0	0.00
10)Prostate adenocarcinoma (PRAD)	32	0	0	0.00
11)Head and neck squamous cell carcinoma (HNSC)	29	0	0	0.00
12)Lung adenocarcinoma (LUAD)	28	0	0	0.00
13)Skin cutaneous melanoma (SKCM)	20	0	0	0.00
14)Thyroid carcinoma (THCA)	17	0	0	0.00

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P12.139C
Circulating plasma miRNAs expression alterations as promising marker in discrimination of EGFR mutation status in NSCLC patients

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Presently, the only predictive biomarker of response to EGFR-TKI therapy in non-small cell lung cancer (NSCLC) patients is the EGFR mutation status. The aberrant expression of miRNAs play a key role in lung carcinogenesis. Herein, we evaluated the potential diagnostic usefulness of several circulating plasma miRNAs expression under different normalization approaches that might have discriminative value for EGFRm+ and EGFRm- NSCLC patients. Total RNA was extracted from 100μL of plasma; material with hemolysis was excluded. Expression of miR-504, miR-122, miR-195, miR-10b, miR-21, and UniSP6 (extraction efficacy control) in plasma of 60 non-squamous NSCLC patients (31 patients EGFR+ in paired tumor tissue and plasma specimen) was investigated using RT-qPCR. Similar procedure was applied for *in vitro* NSCLC cell lines (HCC4006, PC-9, H1975, and H2347). Next, association between circulating miRNA expression and EGFR mutation status was analyzed according to different data normalization approaches (miR-16 and miR-191 as normalizers). Only plasma miR-504 expression was significantly associated with EGFR mutation status in NSCLC patients regardless the normalizer used ($p = 0.0158$ and $p = 0.002$; for adenocarcinoma patients $p = 0.0004$ and $p = 0.001$; for miR-16 and miR-191 normalization, respectively). The highest discriminatory power of circulating miR-504 was shown for patients with exon 19 deletions versus wild-type EGFR normalized to miR-191 (AUC=0.807 $p < 0.0001$). Aforementioned relations were not observed for *in vitro* cell lines. Our study demonstrated the feasibility and potential diagnostic value of miR-504 expression analysis in plasma for discrimination between EGFRm+ and EGFRm- NSCLC patients. However, the normalization strategy is of key importance, strongly impacting circulating miRNA analysis outcome.

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P12.140D

Aggressive prostate cancer development might be under the regulation of both Tregs and miRNAs

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Introduction: The progress of prostate cancer comprises complex contemporaneous tumor developmental events in diverse stages are still yet to be clarified. miRNAs might accompany to balance between regulatory (FOXP3+) and cytotoxic T cells in tumors. Here, we investigated miRNAs and FOXP3 expressions in patients with prostate cancer spectrum.

Material and Method:38 prostate cancer patients enrolled within two groups as having Gleason Score up to 7; 8 or more and 19 benign prostate hyperplasia (BPH) controls. 12 miRNAs expressions were analyzed by real time PCR from paraffin embedded prostate tissue samples. Correlation analyses were made between serum PSA levels, immunohistochemical staining of CD3, CD4, FOXP3 and miRNA expressions.

Results: We found, hsa-let7c-3p significantly 1,52 (p =-0,018) and 1,84 ($p = 0,0095$) fold down-regulated whereas, miR-141-3p was significantly 2,36 ($p = 0,0006$) and 2,24 ($p = 0,001$) fold upregulated in the prostate cancer patients compared to BPH in group 1 and group 2, respectively. Only CD4 ($p = 0,004$) and PSA ($p < 0,001$) have statistically significant differences among groups when compared to BPH. The Treg marker FOXP3 expressions were significantly correlated with miR-143-p, miR-221-3p, hsa-let7c-3p and miR-17-3p expressions. No significant correlations were found between CD3, CD4 and miRNA expressions or between PSA and FOXP3 expressions.

Conclusions: We for the first time reported significantly altered expressions of miRNAs (miR-let7c, miR221, miR-146a, miR-141, miR-143, miR17) and correlations between Treg marker FOXP3 in the prostate cancer patients suggesting that prostate cancer progression might be under the regulation of both Tregs and miRNAs.

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P12.141A

Mitochondrial DNA mutation analysis in molecular subtypes of breast cancer

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Introduction. Breast cancer (BC) is the is the commonest cancer type in women worldwide. Many studies have suggested that variants in mitochondrial genome (mtDNA) are risk factors to develop BC, are associated with severity, relapse or treatment response. To know the landscape of the mtDNA mutations in BC tumors from Mexican women we analyzed mtDNA from paired peripheral blood (PB)-tumor. Materials and methods. We included 39 tumor matched in PB samples from Mexican women with BC. Tumors were classified immunohistochemically and molecularly (PAM50). The entire DNAmt was sequenced through the MiSeq platform (Illumina) and using two overlapping primer sets. Results. 38.5% of the tumors were luminal A (LA), 25.6% luminal B (LB), 10.3% HER2, 10.5% basal (B) and 15.4% normal-like (NL). A total of 349 and 364 variants were identified in SP and tumor, respectively, of which, 225 variants were shared among both tissue. 139 somatic mutaciones were identified in tumors, being the single base substitutions the most common (94%) and the remainder variants were insertions (3%) and deletions (3%). The CYB gene showed the highest number of somatic mutations (18.7%) in comparison with other mitochondrial genes. Stratification analysis by molecular subtype showed that HER subtype displays the highest number of mutations (average: 11 ± 8.2) than the other tumor subtypes. Conclusions. mtDNA from HER subtype showed the highest mutation number suggesting that there is an association between the rate of DNAmt mutations with the molecular subtype of BC and with the prognosis of the disease. Acknowledgments: CONACyT FOSISS 2016-272618

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P12.142B

Mitochondrial DNA (mtDNA) haplogroups and hypervariable region I (HVI) mutations in a cohort of Sri Lankan sporadic breast cancer patients - A preliminary analysis

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Introduction: Breast cancer is the commonest female cancer, globally, as well as in Sri Lanka. Several mtDNA mutations are reported to be associated with breast cancer, but there are no data for Sri Lanka.

Methods: Thirty patients with sporadic primary breast cancer and their age, BMI and menopausal status matched controls were studied. Macro-haplogroups M and N were determined using coding region SNP based PCR-RFLP. A 900 bp region covering the hypervariable region I of mtDNA was PCR-amplified, sequenced and analysed.

Results: 18 patients and 17 controls belonged to the M macro-haplogroup and the remainder to the N macro-haplogroup. In total 72 and 45 mutations were identified in the patients and controls respectively, with 38 and 15 of them occurring exclusively in each group. Prevalence of mutations previously reported to be associated with breast cancer, namely T16189C, T16519C, T16311C and C16207T were not significantly different between patients and controls. Exclusive mutations seen in 23 patients were distributed as follows: 05 (N=3), 04 (N=2), 03 (N=3), 02 (N=7) and 01mutation (N=8). Some patients had more than one exclusive mutation with the highest number co-existing being five.

Conclusion: Mitochondrial DNA D-loop mutations previously unreported to be associated with breast cancer observed in the present study require further analysis especially since several such mutations co-exist in some patients. Prevalence of HVI mutations reported to be associated with breast cancer or M and N macro-haplogroup status did not significantly differ between patients and controls in present study.

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P12.144D

RET Genotype & MEN2 Phenotype correlation in a large Indian Medullary Thyroid Carcinoma (MTC) cohort & influence of SNPs in 3 genetic pathways on MTC behavior

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Introduction: MTC is an aggressive cancer of Thyroid parafollicular cells. Around 25% MTC cases occur as Multiple Endocrine Neoplasia Type2 (MEN2) syndrome due to germline mutation in RET protooncogene. However phenotypic heterogeneity in individuals harboring the same

RET mutation suggests the role of additional genetic events in MTC. The aim is to identify novel and recurrent germline RET mutations in a cohort of 400 Indian MTC cases and to study the role of 13 different SNPs in genes of distinct pathways on MTC behavior.

Materials and Methods: Germline RET mutation analysis was performed on genomic DNA by Sanger/Next Generation Sequencing. For SNP genotyping RFLP approach was used followed by correlation of SNP genotype with different clinico pathological parameters of patients.

Results: Pathogenic germline RET mutations were identified in 62 families (120 mutation carriers). Double mutation in RET was seen in 3 families & novel mutation in 1 family. In 3 cases with classical MEN2B phenotype Sanger Sequencing could not identify RET mutation. Whole Exome NGS analysis identified M918T mutation in RET indicating allele dropout on Sanger Sequencing. Three of the 13 SNPs studied (Cyp1A1m1, CDKN2A, NAT2) showed significant protective association with lesser metastatic spread and calcitonin levels.

Discussion: This is the first large report on genotype-phenotype association in Indian MTC cohort revealing several distinct associations with double or novel RET mutation and modifier effect of few SNPs on MTC clinical outcome. The not so infrequent occurrence of allele dropout is highlighted in this study.

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P12.146B
Chromothripsis 18 in multiple myeloma patient with rapid extramedullary relapse

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Background: Catastrophic chromosomal event known as chromothripsis was proven to be a significant hallmark of poor prognosis in several cancer diseases. While this phenomenon is very rare in among multiple myeloma (MM) patients, its presence in karyotype is associated with very poor prognosis.

Case presentation: In our case, we report a 62 year female patient with rapid progression of multiple myeloma (MM) into extramedullary disease and short overall survival (OS=23 months). I-FISH investigation revealed presence of gain 1q21 and hyperdiploidy (+5,+9,+15) in 82% and 86%, respectively, while *IgH* rearrangements, del(17)(p13) and del(13)(q14) were evaluated as negative. Whole-genome profiling using array-CGH showed complex genomic changes including hyperdiploidy (+3,+5,+9, +11, +15,+19), monosomy X, structural gains (1q21-1q23.1, 1q32-1q44, 16p13.13-16p11.2) and losses (1q23.1-1q32.1; 8p23.3-8p11.21) of genetic material and chromothripsis in chromosome 18 with 6 breakpoint areas. Next-generation sequencing showed a total of 338 variants with 1.8% (6/338) of pathological mutations in *NRAS* (c.181C>A; p.Gln61Lys) or variants of unknown significance in *TP53, CUX1* and *POU4F1*.

Conclusions: Our findings suggest that presence of chromothripsis should be considered as another important genetic hallmark of poor prognosis in MM patients and utilization of genome-wide screening techniques such as array-CGH and NGS improves the clinical diagnostics of the disease. This project was supported by MUNI/A/0824/2017.

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P12.148D
High prevalence of pathogenic *PTEN* germline mutations in population data

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Introduction: *PTEN* Hamartoma Tumour Syndrome (PHTS) is a severe inherited cancer risk syndrome –including malignant and benign neoplasms, overgrowth and autism– caused by germline mutations in *PTEN*. The prevalence of pathogenic germline *PTEN* mutations is unclear, and estimated at ~1/200,000. The aim of this study was to assess the population prevalence of pathogenic *PTEN* mutations.

Methods: The prevalence of pathogenic *PTEN* mutations was assessed using the genome Aggregation Database (gnomAD) and Exome Aggregation Collaboration (ExAC) database, including 138,632 and 60,706 individuals, respectively.

Mutations were classified as pathogenic (i.e. multiple times reported as pathogenic in ClinVar and/or truncating variants) or predicted pathogenic (i.e. CADD score >25).

For each group, the combined allele frequency (AF) was determined.

Results: In gnomAD, 13 pathogenic (10 variants, AF=2.62e-5) and 14 predicted pathogenic (12 variants, AF=2.69e-5) *PTEN* mutations were detected. In ExAC, 5 pathogenic (5 variants, AF=4.12e-5) and 6 predicted pathogenic (5 variants, AF=4.94e-5) mutations were identified. When in ExAC cancer patients (i.e. TCGA data) were excluded to minimize selection bias, 2 pathogenic (2 variants, AF=1.88e-5) and 5 predicted pathogenic (5 variants, AF=4.72e-5) mutations were identified. This suggests a mutation prevalence of 8-36/200,000.

Conclusion: The prevalence of pathogenic germline *PTEN* mutations is 8-16 times higher than expected based on current estimates. When including predicted pathogenic mutations, this was 21-36 times higher.

This data substantiates that many PHTS patients are still unrecognized, which might be related to varying disease penetrance. With the increasing use of gene panels, this data provides relevant expected detection rates in unselected patients.

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P12.149A

NAT2 polymorphisms are risk factor to acute lymphoblastic leukemia in children from Mexico

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Acute lymphoblastic leukemia (ALL) is the leading cause of pediatric cancer worldwide that display a disparity incidence among ethnic groups. Mexican is one of the most affected population, which also has a high childhood mortality rate due to ALL. Evidences suggest that single nucleotide polymorphisms (SNPs) in genes involved in the metabolism of xenobiotics, such as N-acetyltransferase 2 (*NAT2*) gene, could contribute to the ALL risk. To know wether SNPs in *NAT2* gene are associated with ALL in Mexican children we performed a case control-study. We included 311 (152 cases and 159 controls) individuals aged <17 years old. Cases were newly diagnosed and risk classified base on National Cancer Institute (NCI) criteria. DNA was extracted from peripheral blood and saliva and genotyping was performed using Taqman probes. No statistical differences were detected to rs1799930 SNP between cases and controls, but rs1801280 (OR 2.2, CI 1.6-4.8, p = 4.4-07), rs1789929 (OR 3.53, CI 2.58-4.84, P= 9.9-16), rs1208 (OR0 5.18, CI 3.77-7.10, P= 8.23-26) and rs1799931G (OR=2.48, CI 1.72-3.57, P= 6.08-7) were associated with ALL. In addition the rs1041983 showed the TT genotype (OR 2.048, CI 1.047-4.0, P= 0.03) increased the risk to this malignancy. Our results suggest that SNPs in *NAT2* confer susceptibility to develop ALL in children from Mexico.

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P12.150B

Coexistance of APC and NF1 pathogenic mutations in the same patient

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Introduction: Type I Neurofibromatosis is a rare genetic disease that causes benign nerve tumors, and cutaneous and skeletal manifestations. Mutations in the NF1 gene have been found to cause the disease. Familial Adenomatous Polyposis (FAP) is an uncommon hereditary disease characterized by the appearance of more than a hundred adenomatous polyps. Mutations in the gene APC greatly increase the risk of suffering FAP. Mutations in either of these genes is uncommon, much more so the simultaneous mutation of both genes in the same patient

The case of a 20-year-old patient with rose-colored polypoid lesions in the dorso-lumbar region associated to a possible type I neurofibromatosis is presented. Her clinical history stood out because of a total colectomy to prevent

FAP (several cases of polyposis colorectal cancer were described in her maternal line), several surgeries to remove trichilemmal and epidermic cysts in different locations and an abdominal laparotomy to remove a desmoid tumor.

Material and Methods: DNA extracted from peripheral blood and from each type of tumor underwent heteroduplex analysis

Results: In NF1, a pathogenic alteration (c.5418_5422delGGGC/ p.Q1806QfsX), which generates a truncated protein and causes neurofibromatosis, was found in DNA extracted from the neurofibroma but not from peripheral blood. In APC, a pathogenic mutation (c.3783_3785delTT/p.T1261TfsX), which generates a truncated protein that increases the risk of FAP, was found. Neither of these mutations had been previously described

Conclusion: The patient presented two rare pathogenic mutations in genes responsible for diseases with unrelated phenotypes

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P12.151C

Schwannomatosis associated schwannomas show a different *NF2* mutational spectrum compared to Neurofibromatosis type 2 patients

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Introduction: Two genetically distinct entities, Neurofibromatosis type 2 (NF2) and schwannomatosis, predispose to development of multiple schwannomas. Thus far, two genes are involved in schwannomatosis predisposition: *SMARCB1* and *LZTR1*, both located on the long arm of chromosome 22. *NF2* gene, responsible for NF2, is somatically involved in the four hits/three steps mechanism described in schwannomatosis-associated schwannomas. However, the mutational spectrum of *NF2* gene in schwannomatosis tumors is not well defined.

Materials and Methods: We analysed *NF2* gene in 116 schwannomas from 46 schwannomatosis patients with or without *LZTR1* or *SMARCB1* germline mutation, comparing this mutational spectrum with the one of 117 NF2 patients and 25 mosaic NF2. In schwannomas we also assessed LOH.

Results: We demonstrated a statistically significant increase of indel mutations in schwannomatosis-associated tumors versus the germinal ones.

Conclusions: We found a different spectrum of *NF2* somatic mutations in the schwannomatosis-associated schwannomas compared to the germinal mutations found in NF2 patients, as already demonstrated in sporadic schwannomas. The preponderance of indels as somatic mutations and of single mucleotide substitutions as germline ones, suggest the existence of distinct mechanisms of mutagenesis during mitosis and meiosis. This work was funded by grants from Ministero della Salute and Istituto Toscano Tumori.

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P12.153A

Fifty first tests and beyond: a real world experience of cancer genetic testing in a high risk cancer genetic clinic of a university based urban hospital in Thailand, an upper middle income country

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Introduction: Cancer genetic testing (CGT) in low- to-middle-income countries is less accessible and has mostly been done in the research. We reported here the real-world situation in a high risk cancer genetics clinic (CGC) at a tertiary-center in Thailand.

Methods: We reviewed cases at the high-risk CGC at the Chulalongkorn Hospital in 2017.

Results: Of 69 cases, fifty cases (50/69=72.5%) underwent CGC. The indications for patients tested were personal history of cancer (47/50=94%). Only three unaffected probands with family history of cancers (3/50=6%) were tested. Breast (20/50=40%) and ovarian (6/50=12%) cancers were the indication for the majority of tested patients. We identified fifteen pathogenic variants (PV) or likely PV in *BRCA1*(5),*BRCA2*(3),*APC*(2),*MLH1*(1), *FANCA*(1),*PTEN*(1),*NF1*(1), and monoallelic PV in *MUTYH*(1) in 14 patients (14/50=28%: one patient harbored 2 PV in *BRCA1* and *NF1*). We identified eleven variants of unknown significance in eleven patients (11/50=22%).4 patients underwent prophylactic surgery after testing. Nineteen cases refused(8) or were not offered receiving (clinical diagnoses (2), or in the low risk group (9)) the CGT. The reasons of refusal were financial concerns and the perception of no benefit to themselves. The median age was 40 years old compared to 47 years old in the tested patients. 36.8% (7/19) of patients were unaffected.

Discussion: We presented the CGT in the high risk CGC in the real world setting since the more affordable CGT has

been offered in the last few years. Patients refused or not-offered to be tested were younger and were more unaffected cases than the tested group.

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P12.154B

Pathogenic variants (PVs) detection in a 19-gene core panel and yield of opportunistic screening in *BRCA1/2* and MMR genes in a cohort of 1121 hereditary cancer patients

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Background: Multigene panels provide a powerful tool for analyzing several genes simultaneously and identifying cancer susceptibility beyond the suspected clinical phenotype. We evaluated the PVs frequency in customized pre-defined panels according to phenotype and extended the analysis to our 19-gene research panel. We also investigated the yield of opportunistic screening in the *BRCA1/2* and MMR genes in all patients.

Patients: Overall, 1121 unrelated probands underwent multigene testing with customized pre-defined panels according to their phenotype in addition to *BRCA1/2* and MMR genes, and a 19-gene research.

Results: Overall, 1015 female and 106 male were studied, mean age at cancer diagnosis was 47 years old, 579 had breast cancer (BC), 258 ovarian cancer (OC), 124 colorectal cancer, and 27 were unaffected. A BC, OC, or BC/OC panel was requested in 66% and a HNPCC panel in 12%. One hundred and fifty-one (13%) probands carried at least one PV with the customized diagnostic panel. All *BRCA1/2* carriers fulfilled BC, OC or BC/OC criteria, while among the MMR carriers, 50 (89%) PV were identified in the HNPCC panel and 6 (11%) were opportunistic, all 6 in *MSH6*. The 19-gene research panel provided 22 (2%) additional PV beyond the customized panel according to the clinical phenotype: 5 *BARD1*, 4 *NBN*, 3 *BRIP1*, 3 *ATM*, 2 *CHEK2*, 2 *RAD51C*, 2 *RAD51D*, 1 *CDH1*.

Conclusions: The yield of PV detection in different actionable genes identified by multiplex testing is clinically relevant. Eleven percent of MMR mutation carriers (all carrying *MSH6* PVs) were identified through opportunistic screening.

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P12.155C

***NIPBL* pathogenic variant in a Cornelia de Lange Syndrome patient with Acute Lymphoblastic Leukemia**

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Background: Cornelia de Lange syndrome (CdLS) is a rare genetic disorder characterized by pre- and post-natal growth retardation, mental retardation, facial dysmorphism and upper limb abnormalities. The main causes of the disease are mutations in the *NIPBL*, *SMC1A*, *SMC3*, *HDAC8* and *RAD21* genes, which encode proteins of cohesin complex. Mutations in the cohesin genes have recently been identified in AML, CML and myelodysplastic syndromes.

Objectives: Here, we report the description of the first case of a CdLS pediatric patient who developed precursors B Acute Lymphoblastic Leukemia (BCP-ALL). Furthermore, we investigated the presence of cohesin genes variants in pediatric ALL patients not affected by CdLS.

Results and Conclusions: At disease onset, patient did not present any prognostically relevant cytogenetic abnormality and was enrolled to high risk treatment group for MRD analysis. Through NGS Trusight Pan-Cancer analysis, we identified variants in cohesin genes, in particular in exon 46 of *NIPBL*, in heterozygosity. This variant is a new mutation that causes frameshift and a premature stop codon. This anomaly was confirmed on the patient's bone marrow DNA both at diagnosis and in remission of leukemia and in a buccal smear sample. Both parents and brother are negative, phenotypically normal and not affected by hematological diseases. We further analyzed 86 pediatric BCP-ALL cases considering *NIPBL*, *RAD21*, *SMC1A*, *SMC3*, *STAG2*. We detected 36 variants, identifying recurrent known variants in addition to 10 novel variants in cohesin genes, mainly affecting *NIPBL*, which seems peculiar of ALL, since it has never been reported as altered in AML.

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P12.156D

The allelic frequency for S and Z mutations in the SERPINA1 gene in NSCLC patients from Poland

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Background: Alpha-1 antitrypsin (AAT) is encoded by the highly polymorphic *SERPINA1* gene. Mutations in this gene can lead to AAT deficiency (AATD) which is a risk factor for number of chronic lung disorders. The causative link between AATD and non-small cell lung cancer (NSCLC) is controversial due to insufficient research data. We evaluated the frequency of major pathogenic *SERPINA1* alleles among Polish NSCLC patients.

Methods: blood samples were collected from 468 NSCLC patients. The serum AAT concentration was measured by nephelometry and the phenotype was analyzed by isoelectric focusing. The *SERPINA1* variants were identified by DNA sequencing. The frequency of *SERPINA1* alleles was estimated by means of Hardy-Weinberg equilibrium.

Results: 446 out of 468 (95.2%) NSCLC patients presented normal Pi*MM phenotype. AATD alleles were found in 22 patients. 10 (2.1%) carried Pi*S allele, 10 (2.1%) - Pi*Z allele, and 3 (0.6%) - Pi*F allele. Accordingly, the frequency of major AATD alleles was: Pi*S 10.7/1000 (95%CI:4.1-17.3) and Pi*Z 10.7/1000 (95%CI:4.1-17.3). The mean serum AAT concentration in NSCLC patients was 180 mg/dL in Pi*MM individuals and 148 mg/dL in AATD allele carriers.

Conclusions: The frequency of Pi*S and Pi*Z alleles in NSCLC patients differ noticeably from the actual data of general Polish population study (Pi*Z 13.7/1000 and Pi*S 7.6/1000): the frequency of Pi*Z allele is lower whereas the frequency of Pi*S is higher than normal reference values. The NSCLC patients who carry AATD allele do not present reduced AAT concentration in blood as compared to the reference values from population studies.

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P12.157A
Use of molecular identifiers in targeted NGS to detect mutations below one percent allele frequencies in circulating cell-free DNA

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The growing use of liquid biopsy for early detection and monitoring of disease necessitates accurate variant detection at <1% allele frequencies due to a low population of disease DNA within circulating, cell-free DNA (cfDNA). Reliable, low-frequency variant detection by next-generation sequencing (NGS) is challenging due to background noise from PCR and sequencing errors. We employed molecular identifiers (MIDs) to uniquely label individual DNA molecules prior to amplification, facilitating the distinction of true variants from PCR and sequencing errors. We incorporated MIDs in both our Accel-Amplicon library prep that uses multiplex PCR for targeted NGS and our Accel-NGS 2S whole genome library prep followed by targeting with hybridization capture using an 800kb pan-cancer panel. We performed low frequency spike-in experiments at <1% allele frequencies. We prepared MID libraries from amplicon panels including a 17 amplicon EGFR pathway panel and a 104 amplicon SNP panel. Deep sequencing to >30,000x was done to maximize MID family size (number of PCR duplicates) and optimize generation of a consensus sequence. This analysis identified all known variants present at 1%, 0.5%, and 0.25% allele frequencies. Next, the targeted 2S libraries were prepared with low-frequency spike-in samples, sequenced to >8000x, and all known variants at 1% and 0.5% allele frequencies were maintained in the consensus data. In both cases, the number of false positives was reduced, resulting in improved specificity. This study highlights the ability of MID technology to enable low frequency variant detection, critical to track known variants and identify novel pathogenic mutations in cfDNA samples.

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P12.158B
Investigation of the effect of MET gene expression alteration on oral cavity tumors

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Head and neck cancers, which constitute 2-5% of all malignancies worldwide and in our society, can be diagnosed early by increasing the use of endoscopy and have a high survival rate with appropriate treatment. There is no definitive tumor marker used in the diagnosis and follow-up of head and neck cancer. Genetic factors involved in the formation of head and neck cancers have not been identified. The MET gene encodes the proto-oncogen MET, a member of the protein receptor tyrosine kinase family. Mutations in these gene have been associated with papillary renal cell carcinoma, hepatocellular carcinoma, and various head and neck cancers. In this study, we aimed to investigate the expression changes of MET gene in oral cavity cancers which are thought to be effective in many cancers. RNA isolation was performed from tumoral and normal tissue specimens stored in paraffin blocks of 30 patients under the age of 40 years, with a mean age of 32-41 and who did not smoke and whose oral cavity tumors were present between January 2016 and August 2016. MET gene expression levels were analyzed by real-time quantitative reverse transcription in comparison with normal tissues. Postoperative pathology of all patients was squamous cell carcinoma. According to the results, when compared to normal tissues, the expression levels of MET genes were increased by 8.47 fold in tumor tissue. The obtained data will guide the molecular recognition and mechanism of the disease and provide prognostically valuable information by associating it with recurrence / survival.

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P12.159C

Tumour DNA *BRCA1/2* testing in newly diagnosed ovarian cancer as pre-selection for PARP inhibitor therapy and germline testing is feasible and effective

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Introduction: Ovarian cancer (OC) patients with either somatic or germline *BRCA1/2* mutations may benefit from PARP inhibitor therapy. Tumour DNA *BRCA1/2* testing initiated by the pathologist in newly diagnosed OC patients might effectively identify patients eligible for PARP inhibitor therapy and serve as a pre-selection for germline *BRCA1/2* testing.

Materials and Methods: Newly diagnosed OCs patients form 8 hospitals were included by pathologists. Tumour DNA *BRCA1/2* testing was performed in formalin fixed, paraffin embedded (FFPE) samples using single molecule Molecular Inversion Probe-based targeted next generation sequencing and *BRCA1* Multiplex Ligation-dependent Probe Amplification. Gynaecologists advised patients with a tumour *BRCA1/2* mutation to have genetic counselling and germline *BRCA1/2* testing.

Results: Of 301 consecutive OC patients, 53 (18%) had a tumour DNA *BRCA* mutation of which 56% was diagnosed with a germline *BRCA1/2* mutation. More than half (58%) of the families with a germline *BRCA1/2* mutation did not comply with criteria for germline *BRCA1/2* testing based on family history prior to the OC diagnosis. 81% of all newly diagnosed OC patients were included by the pathologists for tumour DNA *BRCA* testing. Median turnaround time of tumour DNA *BRCA* testing was 14 days. The workflow was positively reviewed by all patients and 83% of the gynaecologists.

Discussion: Tumour DNA *BRCA1/2* testing in all newly diagnosed OC patients as pre-selection for PARP inhibitor therapy and germline testing is feasible and effective. More than half of the patients with a germline *BRCA1/2* mutation would not have been identified by family history.

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P12.160D

BRCA1 AND *BRCA2* GENES MUTATIONAL SCREENING IN FFPE OVARIAN CANCER TISSUES

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Ovarian cancer patients with germline or somatic pathogenic variants in *BRCA* genes benefit from treatment with poly ADP ribose polymerase inhibitors (iPARP1). To select patients who may benefit from these treatments, assessment of the mutation status of *BRCA1* and *BRCA2* in the tumor is required. Tumor *BRCA1/2* testing is more challenging than germline testing as the majority of samples are formalin-fixed paraffin embedded (FFPE), the tumor genome is complex, and the allelic fraction of somatic variants can be low. There is the need for efficient and timely methods to detect both somatic and germline mutations using FFPE tissues and commercially available technology. We used commercial kits to explore all exons and 50bp exon-intron junctions of *BRCA1* and *BRCA2* in next-generation sequencing (NGS) on DNA from 40 FFPE ovarian serous carcinoma tissues. In total, 10/40 patients (25%) carried 11 mutations (9 in *BRCA*1 and 2 in *BRCA*2). Pathogenic variants were confirmed by Sanger sequencing in tumor DNA. One novel frameshift *BRCA*1 mutations was found. The germline or somatic status of the mutations will be assessed in DNA from blood sample or from FFPE non neoplastic tissues. This study evaluates the relevance of standardization in tumor *BRCA* testing particularly when the test results dictate clinical decisions regarding life extending therapies of patients.

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P12.162B
Mir200a, miR200b and miR200c as candidate biomarkers in the diagnosis of ovarian cancer

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Introduction: Ovarian cancer is the fifth most common form of cancer death among women. Developing a fast, reliable blood test would facilitate the early detection of ovarian cancer, which would also contribute to the improvement of survival chances. Screening circulating miRNAs proved to be reliable biomarkers in various cancers. The dysregulation of miRNAs is also known in ovarian cancer, however, only a few publications focus on their diagnostic role.

Materials and Methods: We screened several miRNAs (*miR200a, miR200b, miR200c, miR205, miR483, let7f*) in the plasma samples of healthy ($n = 30$), previously untreated serous epithelial ovarian carcinoma patients ($n = 17$, FIGO stages III or IV) and patients with benign masses ($n = 14$). The relative amount of miRNAs was detected by qRT-PCR.

Results: The expression levels of *miR200a, miR200b* and *miR200c* were elevated in the carcinoma samples compared to the healthy donors ($p < 0.00001$). The level of *miR200a* was also higher in cancerous than in benign samples ($p < 0.01$). However, no significant difference was detected in the case of *miR205, miR483* and *let7f*. Diagnostic accuracy was the highest in the case of *miR200a*: 87.23% with the power area under the curve (AUC) of 0.91 (95% CI=0.79-1). The diagnostic accuracy was also promising in the case of *miR200b* and *miR200c*: 80.85% and 74.47% with AUC 0.82 (95% CI=0.67-0.97) and 0.8 (95% CI=0.64-0.96) respectively. The correlation was relatively high between the test results of *miR200b* and *miR200c*.

Conclusions: *MiR200a, miR200b* and *miR200c* are applicable biomarkers in ovarian cancer.

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P12.163C
PALB2 c.2257 C&gtT mutation is a Greek founder and is associated with early breast cancer diagnosis

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Introduction: Germline mutations in *PALB2* (Partner And Localizer of BRCA2) are rare, attributing for approximately 1–2% of familial breast cancer (BrCa) cases; their frequency can be possibly influenced by founder effects. Deleterious *PALB2* variants are clinically important, predisposing for breast, pancreatic and possibly, ovarian cancer. Ten, apparently unrelated, Greek families carried the *PALB2* c.2257C>T (p.Arg753*) mutation; we therefore sought to

clarify its possible founder effect and to investigate associations with cancer diagnoses.

Methods: Genotyping for the mutation was pursued either by multi-gene panel testing, Sanger sequencing or Real-Time PCR, followed by haplotype analysis of mutation carriers. Genotyping was performed on 2791 breast (median age 47.8 years ± 11.23), and 436 ovarian cancer (median age 52 years, ± 13) patients. Approximately one third of patients tested had family history (at least one relative diagnosed with breast, ovarian or pancreatic cancer).

Results: The mutation prevalence was 0.36% (10/2791) and 0.23% (1/436) in breast and ovarian cases, respectively. Targeted testing on family members revealed five more carriers. All eleven families had strong family history, while pancreatic cancer diagnosis among close relatives was reported in two families. Median age at breast cancer diagnosis among carriers was 41.8 years (range 33 – 58), suggesting association with earlier breast cancer diagnosis ($p = 0.02$). Haplotype analysis revealed a common haplotype spanning 0.7Mb.

Discussion: Herein, the *PALB2* c.2257 C>T mutation is shown to be a Greek founder mutation, statistically significantly associated with earlier breast cancer diagnosis, while families had strong family history of cancer, specifically enriched for pancreatic cancer incidence.

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P12.164D

Cell-free circulating tumor DNA monitoring in pancreatic cancer patients

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer death worldwide. 80-85% of patients are diagnosed at an advanced stage since clinical presentation often occurs very late in the disease process. In this study, we evaluated cell-free circulating tumor DNA (ctDNA) of 21 PDAC patients before treatment and under chemotherapy in serial plasma samples.

Material and Methods: Three serial plasma samples per patient were obtained. Library preparation of 63 liquid biopsies was conducted using Illumina TruSight Tumor 15 (TST15) and the brand-new AmpliSeq for Illumina Cancer HotSpot Panel, which covers 50 cancer-related genes. Treatment response was evaluated via computer tomography before and 3 months after therapy.

Results: Technically, all 63 samples were sequenced successfully with both library kits. NGS resulted in average 3.65 Mio passed filter reads for TST15 and 1.07 Mio for Ampliseq with mean amplicon coverages of 21718 and 4368, respectively. The ctDNA mutation frequencies were between 1.2% and 21.7%. 8/21 patients showed *KRAS* and/or *TP53* driver mutations. Concerning correlation with therapy responsiveness, computer tomography and cfDNA analysis revealed concordant results in 17/21 patients.

Conclusions: This study demonstrates that ctDNA testing is technically feasible for high throughput sequencing and therefore has great potential as a noninvasive monitoring tool for PDAC patients. Nevertheless, refinement of the cancer gene list is necessary since several cases revealed no mutation in the 51 analyzed genes. The discordance of genetic and clinical data in some patients suggests that a deeper biological understanding of this tumor entity is required.

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P12.165A

Polymorphic pre-miR-146a and synergistic action of all of its products on *NTRK2* gene in Papillary Thyroid Carcinoma

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Numerous studies confirm the deregulation of microRNAs in various human cancers, including Papillary Thyroid Carcinoma (PTC). The G/C heterozygosity in rs2910164 in miR-146a-3p predisposes to PTC and occurs as a somatic mutation in thyroid tumors. Deleterious function of rs2910164 results from the fact that the SNP is located in the seed region of miR-146a-3p, thus heterozygous carriers of the SNP produce 3 mature miRs: miR-146a-5p, miR-146a-3p(G) and 146a-3p(C). Interestingly, there is only one gene (*NTRK2* - involved in control of differentiation and programmed cell death), identified in silico as concertedly targeted by the three isoforms. The aim of this study was to analyze the interaction between all the miR-146a isoforms

and *NTRK2*. The analysis conducted in 60 pairs of PTC tumor and adjacent control tissue showed that the expression of *NTRK2* is significantly decreased in PTC, with even higher reduction in patients heterozygous for rs2910164 (61% decrease in 84% of tumors, $p = 6.25^{-4}$). The analysis of miR:*NTRK2* interaction in luciferase assay confirmed that all the polymorphic miR-146a products interact with target gene and this effect is additive, giving a 26%($p = 3.49^{-11}$) reduction of luciferase activity by the synergistic binding of all isomiRs compared with 21%($p = 1.96^{-6}$) for the main miR-146a-5p isoform.This is the first functional study of the synergistic effect exerted by polymorphic miRNAs produced from a single precursor. The results may give a strong basis for better understanding of the role of miR-146a and *NTRK2* gene in development of PTC. This work was supported by the Polish National Science Centre Grant DEC-2012/07/N/NZ2/01333

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P12.166B

The incidence of occult ovarian neoplasia and cancer in *BRCA1/2* mutation carriers after the bilateral prophylactic salpingo-oophorectomy (PBSO): a single center prospective study

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Objective: to evaluate the incidence of occult neoplasia in specimen collected during PBSO and to determine the significance of this operation in *BRCA*1/2 mutation carriers.

Methods: Between January 2010 and October 2016 a total of 564 new germline *BRCA1/2* mutation positive women were identified in VULSK. Laparoscopic PBSO was performed for 71 eligible women, who opted for this procedure and were included in this perspective study. Fifty nine women (83,1%) were *BRCA1* and 12 (16,9%) were *BRCA2* mutation carriers; study patients harboured 22 different *BRCA1/2* germline mutations.

Results: STIC was diagnosed in seven (9.85%; 95% CI 4.58 - 19.26) women and occult ovarian cancer (OC) was found in four (5.6%; 95% CI 1.80 - 14.03) women. The median age of women that were diagnosed with STIC or OC at the time PBSO was 45,9 years (± 6,68; P=0.7314), the youngest patient being 42 and the oldest 64 years. All 11 women who were diagnosed with STIC or OC had *BRCA1* mutations. Interestingly, STIC was detected in 6 carriers (out of 27) of exclusively *BRCA1* c.4035delA mutation (22.2%; 95% CI 10.26 - 41.10), a common Baltic founder mutation. The difference between STIC detected in c.4035delA (6/27) and other mutations carriers (1/44) was statistically significant (P=0.0105).

Conclusion: Detection rate of precursor-only lesions (STIC) in our study (9.85%) was slightly higher than previously reported (5-8%). We found statistically significant enrichment of *BRCA1* c.4035delA mutation carriers in STIC group, which may indicate that c.4035delA mutation carriers may have an increased tumorigenesis rate in fallopian tubes.

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P12.167C

Clinical validation of a gene panel refines diagnostics and tailors personalised treatment in pediatric cancer patients

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Introduction: Despite the remarkable improvement in survival, cancer remains the leading cause of mortality in childhood. In this work, we introduce the development and clinical validation of a pediatric solid tumour gene panel, based on NGS. This gene panel allows the characterisation of somatic and germline mutations in solid tumours in primary or relapsing malignancies. The aim of the study is to provide clinically relevant information in diagnostics, prognostics and personalised therapy.

Methods: An NGS multigene panel of 254 full-sequence genes is presented in this work. Tumour and blood paired samples were sequenced on a NextSeq 500 System (Illumina) according to the manufacturer's instructions (Agilent, SureSelect QXT protocol). FASTQ files were analysed by means of a self-developed pipeline for the detection of somatic variants, indels and CNVs.

Results and Conclusions: A set of 40 samples were analysed in this study. First, we worked with fresh-frozen samples, belonging to a retrospective cohort of 16 primary tumours including the ten most-frequent pediatric tumour subtypes. In a second stage, we included 24 patient cases which directly benefited from an accurate diagnostic and personalised treatment based on the present genetic findings. Finally, we screened matching formalin-fixed paraffin-embedded (FFPE) tissue when this was available. Here, we were able to validate and fine-tune the performance of our gene panel in low-quality fragmented

DNA. Future studies include the testing in tissue obtained from minimally invasive tests such as liquid biopsy. This is of special interest in those pediatric patients with unresectable cancers or otherwise challenging to biopsy.

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P12.168D

Peutz-Jeghers syndrome caused by a *de novo* 19p13.3 deletion containing *STK11* gene detected by exome sequencing

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Introduction: Peutz-Jeghers syndrome is an autosomal dominant disease associated with gastrointestinal polyposis and mucocutaneous pigmentation. It usually results from germline point mutations in *STK11* gene, large deletions are rare. Patients with 19p13.3 deletions encompassing *STK11* gene have been reported to constitute a distinctive phenotype of intellectual disability, hypotonia and dysmorphic features.

Materials and Methods: We are presenting a 12-year old girl with mild intellectual disability, tremor in hands, clumsy gait with muscle weakness and hypotonia, positive Gowers' sign, dysmorphic facial features and mucocutaneous pigmentation. At first NGS gene panel (~4800 genes) was sequenced, which showed no alterations associated with her phenotype. Due to muscular phenotype muscle biopsy was done, which showed lipid accumulation. In suspicion of inherited myopathy trio exome analysis was performed and unexpectedly copy number algorithm CoNIFER identified a deletion of chromosomal region 19p13.3, which was confirmed by chromosomal microarray analysis (CMA).

Results: The deletion of 19p13.3 (1,152,656-2,120,154) seen on exome analysis and later confirmed by CMA was ~1 Mb long and comprised eight disease associated genes including *STK11*. Neither of the parents carried this alteration.

Conclusions: We present an additional case of 19p13.3 deletion encompassing *STK11* gene and forming a distinctive phenotype. Muscular symptoms, beside hypotonia, are not described before in 19p13.3 deletion patients. Therefore, it is not precisely known which gene in 19p13.3 region is responsible for muscular phenotype. Finding the most efficient diagnostic algorithm for each patient is challenged by large clinical and molecular variability of Peutz-Jeghers syndrome.

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P12.169A

Exome sequencing in a case of pheochromocytoma/paraganglioma-polycythemia syndrome

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Introduction: Germline mutations in *PHD1*, *PHD2* genes and either germline and somatic mutations in *EPAS1* have been detected in some patients diagnosed with the rare pheochromocytoma/paraganglioma with polycythemia syndrome.

Materials and Methods: A man developed symptomatic arterial hypertension at age 29 and was subsequently diagnosed with unilateral pheochromocytoma and multifocal paragangliomas. Polycythemia had been reported as an incidental finding at age 13 and confirmed afterwards. *JAK2* gene sequencing found no mutations. Family history was irrelevant; polycythemia was excluded in the patient's parents and younger brother. Whole Exome Sequencing (WES) was performed on constitutional DNA of the patient and his parents and on DNA from fresh-frozen paraganglioma tissue collected at surgery, using the MedExome library enrichment method (Roche) and the Illumina Next500 platform. Data were aligned and filtered using our internal pipeline. Variants were annotated using ANNOVAR and validated by Sanger sequencing.

Results: WES failed to detect mutations in *EPAS1*, *PHD1* and *PHD2*, or in genes associated to hereditary pheochromocytoma-paraganglioma (*VHL*, *SDHA*, *SDHB*,

SDHC, *SDHD*, *SDHAF2*, *TMEM127*, *MAX*, *KIF1B*, *TCEB1*). Compound heterozygosity for two very rare variants (p.W342X and p.R571H) in *OVGP1* (encoding for Oviductal Glycoprotein 1, expressed in ovary but also in male gonads and in some cancers) were found in patient's constitutional DNA, inherited from the parents, while in the tumor a somatic frameshift variant in *CHST15* (encoding for carbohydrate sulfotransferase 15) was detected.

Conclusions: Clinical manifestations in this patient seem unrelated to known genes; studies are ongoing to assess the potential role of the variants detected.

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P12.170B

Complex analysis of genomic and transcriptomic alterations in tumor tissue associated with presence of various subpopulations of circulating tumor cells in primary breast cancer

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Background: CTCs play major role in tumor dissemination and progression and are one of the key components of metastatic cascade. The aim of this study was to identify signaling pathways associated with presence of CTCs in primary breast cancer (PBC) patients using comprehensive genomics approach.

Methods: This study included 78 patients with PBC. CTCs were detected before surgery by quantitative RT-PCR assays for expression of epithelial (EP) or epithelial-mesenchymal transition (EMT) genes. Total RNA was used for expression profiling by microarray approach and total DNA for breast cancer related gene panel resequencing.

Results: Mutations in BRCA1/2 genes in tumors were more common in patients with EP_CTC compared to patients without EP-CTC in peripheral blood (23.5% vs. 0%, $p = 0.02$), while there were no mutation in specific gene associated with CTC_EMT. Further, 90 genes and 7 miRs were expressed at significantly different levels in EP_CTCs tumors and 199 genes and 13 miRs in CTC_EMT tumors when compared to tumors without detectable CTCs. Moreover 39 overlapping genes and 7 miRs were found to be expressed at significantly different levels in tumors with EP_CTCs and/or CTC_EMT compared to tumors without detectable CTCs.

Conclusions: We identified for the first time various genomic alterations in tumor tissue associated with different CTCs subpopulation in PBC patients. We suppose, that these genomic alterations could play a role in tumor dissemination and might lead to identification of new therapeutic targets.

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P12.171C

The *in-vitro* evaluation of anticancer effect of DMU-212, a novel resveratrol analog, on prostate cancer cells

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Prostate cancer (PCa) is the second leading cause of cancer among men in developed countries. It is known that Epithelial Mesenchymal Transition (EMT) plays a crucial role in the progression and metastasis of PCa. It has been reported that DMU-212, which is one of the resveratrol analogs, induces G_2/M arrest. In our study, we focused on the effect of DMU-212 on the cell cycle, apoptosis, migration, and invasion of prostate cancer cells. The cytotoxicity effect of DMU-212 on LNCaP and PC-3 cells was determined by WST-8 test. While the apoptotic effect of determined IC_{50} dosages of DMU-212 was tested by Annexin V, PI analysis and flow cytometry were used to evaluate its effect on cell cycle. The invasion and migration

of PCa cells were analyzed by "Cell Biolabs CytoSelect 96 well Cell Invasion Assay Kit" and "Wound Healing Assay", respectively. PCR Arrays were assessed 84 EMT-related gene. E-cadherin and vimentin level changes were assessed by immunofluorescence. Additionally, the protein expressions of cyclin B1, E-cadherin, and β-catenin were tested by western blot. It was observed that the IC_{50} dosage of DMU-212 induced apoptosis, reduced the invasion and migration, and induced predominantly G_2/M arrest in LNCaP and PC-3 cells. It was shown that DMU-212 inhibited the EMT by suppressing the WNT signaling pathway, especially, on PC-3 cells. In conclusion, compatible with the hypothesis of that the inhibition of EMT may prevent metastasis in PCa; it is believed that the DMU-212 has a potential role in the treatment of cancer.

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P12.172D

Prostate cancer genetics clinic: First 16-month report of establishing a multidisciplinary service

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Introduction: Recent studies show that over 10% of men with metastatic prostate cancer harbor a pathogenic germline mutation. To address this patient population, the Seattle Cancer Care Alliance established the prostate cancer genetics clinic (PCGC) in order to (1) help identify patients who meet criteria for clinical genetic testing, (2) ensure appropriate oncologic follow up for patients who carry a pathogenic mutation, (3) support cascade testing of at-risk family members and (4) inform and connect patients with research and clinical trial opportunities.

Materials and Methods: From October 2016 to January 2018, 60.9% (42/69) of patients seen in PCGC had germline genetic testing ordered at the time of their clinic visit. Another 23.2% (16/69) of patients presented to the clinic with germline genetic testing already complete.

Results: A total of 5 (11.9%; 5/42) germline mutations that were presumed to be pathogenic were detected in BRCA2(2), HOXB13(1), MSH6(1), and ATM(1). Analysis also showed 9 variants of uncertain significance, and two patients were found to have an incidental finding (i.e. heterozygous for a pathogenic MUTYH mutation). For those previously tested, 11/16 (68.8%) patients were found to have pathogenic mutations: BRCA2(6), CHEK2(2), PMS2(1), BRCA1(1), and TP53(1).

Conclusions: Genetic predisposition to prostate cancer risk was confirmed in 23.2% (16/69) of patients seen as part of the PCGC service. This provides an opportunity to advocate for the clinical importance of germline genetic testing in men with metastatic prostate cancer, not only for their personal oncologic treatment, but also for the identification of at-risk family members.

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P12.173A

Genome editing to map the role of 7p14.3 locus in prostate cancer

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Prostate cancer (PCa), the second most common cancer among men, is a highly heritable molecularly and clinically heterogeneous disease. In this work we focused on the study of germline variants as putative responsible for early somatic events in PCa. *In silico* analysis by our group identified a non-coding polymorphic regulatory element at the 7p14.3 locus that is associated with DNA repair and hormone regulated transcript levels and with a prostate cancer specific subclass with high genomic instability. *In vitro* studies confirmed the enhancer activity of the locus and the binding affinity for two transcription factors (TFs); androgen receptor (AR) and CCAAT/Enhancer Binding Protein (C/EBP) beta (CEBPB). To first proof a functional role of the 7p14.3 locus I deleted 731bp of the genomic area of interest in PC-3 cells by using CRISPR-Cas9. RNA-seq analyses performed upon AR overexpression and/or CEBPB silencing revealed significant deregulation of the transcriptome in edited versus non-edited cells (control cells). To well-characterize TFs binding motifs flaking the locus, I am performing the disruption of AR and CEBPB motifs via CRISPR-Cas9 in prostate cell lines. Moreover, to better study the role of minor allele of the polymorphism on genomic instability, I am currently working on the establishment of isogenic PC-3 cells via CRISPR-Cas9. Once clones with the three possible genotypes are selected, I plan on testing DNA damage response under relevant conditions. This work is a proof of concept of germline predisposition to molecularly distinct cancer subclasses and has the potential to nominate new mechanisms of cancer development.

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HER2 gene amplification in patients with prostate cancer: Evaluating a CISH-based method

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Prostate cancer (PCa) is one of the most widespread malignancies in the world. The role of the human epidermal growth factor receptor 2 (HER2) in the pathogenesis and progression of human PCa remains poorly understood. In contradiction with breast cancer, studies on HER2 overexpression and gene amplification in PCa have produced varying results, although the HER2 oncogene has been implicated in the biology of numerous tumor types, and serves as a prognostic marker and therapeutic target in breast cancer. Technical challenges are considered the main reasons for data discrepancies. Amplification of the HER2 gene has previously been reported in PCa, in which it was associated with tumor progression. The present study aimed to evaluate the prevalence and clinical significance of HER2 amplification in PCa. A total of 32 biopsy samples obtained from human prostate adenocarcinomas were evaluated by chromogenic *in situ* hybridization (CISH) to determine the frequency of patients with HER2 gene amplifications. High copy numbers of HER2 were detected in 19 of the prostate tumors analyzed. The results of the present study suggested that, in patients without amplification of HER2, high levels of prostate-specific antigen or a high Gleason score were not significantly correlated with a high pathologic stage. Furthermore, amplification levels of the HER2 gene were directly associated with pathologic stage in patients with PCa. Therefore, the potential use of HER2 as a prognostic factor or therapeutic target for PCa warrants further study.

N. Sharifi: None.

P12.175C

Liquid biopsy for detection of genomic instability in prostate cancer

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Introduction: According to statistical data, cancer is among the main causes of morbidity and mortality in the Slovak Republic. Since genomic instability plays a crucial role in the malignant progression of prostate cancer, it may be used as a potential biomarker. In current clinical practice in Slovakia, histological examination of tumor tissue is used for diagnosis and staging of cancer. This approach is far from a perfect solution because it requires invasive procedure. In addition, the obtained information is not always sufficiently representative. On the other hand, genomic instability plays a crucial role in the malignant progression of prostate cancer, so it may be used as a potential biomarker. A non-invasive method based on liquid biopsy for detection of genomic instability represented by copy number variations, may lead to more precise diagnosis and may replace traditional, painful procedures on patients.

Materials and Methods: We performed whole genome sequencing analysis of cfDNA from set of patients with positive PSA screening result (10) and healthy individuals (10). We compared sequencing profiles of these groups to identify cancer specific copy number aberrations.

Results: Identified differences between prostate cancer patients and healthy individuals were summarized and further analyzed to trace recurrent patterns.

Conclusions: Our results suggest that prostate cancer specific copy number aberrations can be used as potential non-invasive biomarker. Introduction of this approach to routine clinical practice would be a tremendous contribution to preventing or monitoring of cancer and personalizing anticancer therapy.

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P12.176D

Diagnostic competence of mir-375 and mir-93-5p serum levels in malignant and non-malignant prostate diseases

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Introduction: The study purpose is to examine the diagnostic competence of miR-375 and miR-93-5p serum levels in prostate cancer differential diagnosis. It is also aimed to examine whether there is an interfering situation in the differentiation of chronic prostatitis, benign prostatic hyperplasia (BPH) and prostate cancer, considering the oncogenic and tumor suppressor properties of miRNAs together with the proinflammatory characteristics.

Materials and Methods: 25 Patients with BPH, 10 patients with chronic prostatitis and 33 patients with prostate cancer were included in the study. RNA isolation, cDNA synthesis and qRT-PCR steps were performed with Qiagen branded kits based on SYBR Green method, using the protocol of the commercial company. For statistical analysis, -ΔCt values, obtained from the use of ce-miR-39 Ct values in normalization, were used. Differences between groups were tested by independent sample t test and variance analysis. ROC analysis was performed to calculate the diagnostic competence. "Fold change" calculations were performed online on Qiagen webpage. In all analyzes, alpha error level was accepted as 0.05.

Results: The results are summarized in Table-1.

Conclusions: It was observed that miR-375 and miR-93-5p differ in significantly between malignant and non-malignant disease groups. It was assessed that the sensitivity of miR-375 to differentiate non-malignant diseases from malignant diseases was higher than prostate specific antigen (PSA) and chronic prostatitis may be an interfering condition in BPH and cancer differential diagnosis.

Results

Compared Groups	miRNA	P value	Fold Change	AUC	Specificity	Sensitivity
Malignant-Non-malignant	miR-375	**<0.001**	-4.48	0.781	91%	46%
Malignant-Non-malignant	miR-93-5p	**0.045**	-1.79	0.662	91%	23%
BPH-Chronic prostatitis	miR-375	0.324	–	–	–	–
BPH-Chronic prostatitis	miR-93-5p	0.338	–	–	–	–
BPH-Cancer	miR-375	**<0.001**	-5.60	0.829	91%	56%
BPH-Cancer	miR-93-5p	0.392	–	–	–	–
Chronic prostatitis-cancer	miR-375	0.169	–	–	–	–
Chronic prostatitis-cancer	miR-93-5p	**0.046**	-2.78	0.744	91%	50%

Y. Dulgeroglu: None. **O. Eroglu:** None.

P12.177A

Genetic, pre and post-test findings in a survey of PTEN mutation carriers

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Introduction: Germline heterozygous mutations of PTEN are responsible for hamartoma tumour syndrome (PHTS), a wide spectrum of phenotypes characterised by high tumour risk in diverse tissues. PTEN loss of function also leads to macrocephaly, risk of autism, psychomotor delay (PD) and vascular anomalies.

Methods, Patients and Results: We analysed a cohort of index cases referred to our laboratory for PTEN gene mutation testing (SNV, indel and CNV analysis) : among 151 children referred, 30 were found to have a PTEN mutation, and 47 % were estimated de novo. All positive cases presented with macrocephaly and 91% had a PD. The positive predictive value (PPV) for macrocephaly is 24% (CI28-48) and 23% (CI16-31) for PD/autism.

Adults were referred for Cowden syndrome suspicion (n=122) and presented with diverse clinical findings considered as criteria for the syndrome. The PPV were important for Lhermitte-Duclos syndrome (89%, CI 52-100%), cutaneous manifestations (55%, CI: 36-74), macrocephaly (38%:CI28-48), and intestinal polyps (42%, CI:23-61). Although poorly specific, benign breast lesions (58%, CI: 32-86) and benign thyroid lesions (46%, CI:31-69) show high PPV.

Among 46 adult mutation carriers, post-test examination identified a Lhermitte-Duclos in 3 cases and vascular anomalies were identified in 6 more patients. Two breast and 2 thyroid cancers were also detected within a two-year follow-up. Vascular anomalies were also found in children.

Conclusions: These results confirm the frequency of de novo mutations found in children and emphasize the importance of a systematic search for all features of PTEN syndrome in mutation carriers enabling an appropriate management.

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P12.178B

Bannayan-Riley-Ruvalcaba syndrome due to a new PTEN gene mutation

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Introduction: Bannayan-Riley-Ruvalcaba syndrome is part of PTEN Hamartoma Tumor Syndrome along with Cowden syndrome, PTEN-related Proteus syndrome and Proteus-like syndrome. It is characterized by macrocephaly, multiple noncancerous tumors, and tumor-like growths. 60% of cases are associated with PTEN gene mutations.

Materials and Methods: We present the case of a 15 year old girl we have been investigated since she was 2 years old for macrosomia. She had a spina bifida, right sided facial nerve palsy, and developed deep vein thrombosis. Meantime she undervent a subtotal thyroidectomy for unilateral thyroid adenoma, she had a right sided ovarial cyst resection, and an endovascular correction of a right sided arterio-venous fistula at the transition of sinus transversus and sigmoideus. Kariotyping, Sotos syndrome FISH testing and PTEN gene Sanger sequencing were performed.

Results: She had normal kariotype, Sotos syndrome FISH testing turned out negative. A new heterozygous missense mutation c.2104G>T (p.Val249Gly) was detected in the PTEN gene. In silico analyses predicted it to be deleterious.

Conclusions: PTEN mutations disrupt the mTOR cell signalling pathway, thus leading to a longer cell survival and proliferation. Treatment with mTOR inhibitors should be tested for this cathegory of patients.

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P12.179C

Contribution of *RAD51D* germline mutations in breast and ovarian cancer in Greece

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Introduction: *RAD51D* is a member of the RAD51 protein family and its protein product is known to be involved in the DNA repair mechanism by homologous recombination. *RAD51D* germline mutations have been associated with ovarian (OC) and breast cancer (BC) predisposition. Although the association to OC is established, BC risk conferred by *RAD51D* mutations is still questionable. Our aim was to assess the frequency of *RAD51D* mutations in a large cohort of Greek patients.

Materials and Methods: We screened 609 patients diagnosed with OC, unselected for age or family history and 569 BC patients diagnosed under age 55 with a relative with BC or OC, for *RAD51D* germline mutations. All patients screened by NGS, were previously tested negative for *BRCA1* and *BRCA2* mutations.

Results: We identified four pathogenic mutations in four unrelated individuals with family history of BC and/or OC. Three of the carriers had developed BC while the other one was an OC patient, thus accounting for a mutation frequency of 0.16% in the OC cohort, and 0.53% in the BC cohort. One of the detected mutations is novel (c.738 +1G>A), while the rest had been detected previously (p. Gln151Ter, p.Arg186Ter, p.Arg300Ter). It is noteworthy that among family members of the four carriers, thirteen BC cases were reported and only four OC cases, suggesting a BC association to *RAD51D* mutations.

Conclusions: *RAD51D* should be implemented into the multigene panel testing offered for genetic screening of BC or OC families, since *RAD51D* carriers may benefit from the administration of PARP inhibitors.

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P12.182B

A challenging clinical case. Patient with multiple primary neoplasias and no family history of cancer: Richter syndrome versus Familial Melanoma plus Li-Fraumeni syndrome

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Common risk factors for multiple primary neoplasias are inherited predisposition to cancer; cancer-promoting aspects of lifestyle; hormonal and environmental factors; treatment of the previous primary cancer and increased surveillance of cancer survivors.

A case with multiple primary neoplasias and no relevant family history of cancer was tested to search for a putative genetic etiology of her neoplasias.

The patient under study was diagnosed of endometrial cancer, melanoma, breast cancer and chronic lymphatic leukemia (CLL) at age 58, 60, 76 and 79 years, respectively. Blood DNA was analyzed with a NGS custom panel of 135 hereditary cancer genes (I2HCPv2.2). SureCall-Cartagenia-Agilent tools were used for the bioinformatic analysis. AMG criteria were used for variant classification. Pathogenic variants were confirmed by Sanger sequencing.

We found two pathogenic mutations: *CDKN2A*: c.152dupT;p.V51fs (not previously reported) and *TP53*: c.637C>T;p.R213* (recurrently reported as somatic mutation). The fact that the mutant allele frequency of these two pathogenic variants was around 0.20, suggests the possibility of being somatic mutations as a consequence of an early Richter syndrome not clinically evidenced. This is a CLL form with a highly aggressive phenotype. In this situation, no genetic cause could justify the personal history of cancer. An alternative possibility is that the patient carries two mosaic alleles in genes responsible for Familial Melanoma and Li-Fraumeni syndromes, respectively. Although this scenario is very unlikely, it would explain the previous neoplasias as caused by genetic predisposition.

In some special circumstances, differential genetic diagnosis might be highly difficult. Further studies are needed to clarify this dilema.

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P12.183C

DNA base excision repair in Rubinstein-Taybi (RSTS) cells: insights to cancer predisposition of RSTS patients

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Introduction: The Rubinstein–Taybi syndrome (RSTS) is a rare genetic disease characterized by growth defects, intellectual disability, and an increased tumor formation. RSTS patients carry heterozygous mutation/deletion of the *CREBBP* or *EP300* gene encoding CBP or p300 lysine acetyltransferases, essential proteins playing a key role in transcription and in epigenetic regulation through histone acetylation. In addition, both proteins acetylate p53 and DNA repair factors involved in nucleotide (NER) and base excision repair (BER).

Methods: In order to investigate whether a possible altered DNA damage response (DDR) may concur to RSTS pathogenesis we have analyzed DNA repair efficiency in RSTS lymphoblastoid cells (LCLs). To this end, RSTS cells were treated with potassium bromate, a chemical inducing oxidative DNA damage, and their viability assessed.

Results: RSTS cells were more sensitive than normal LCLs to oxidative DNA damage, but also to agents inducing replication stress such as the Topoisomerase I inhibitor camptothecin. In addition, we have analyzed the acetylation levels of factors participating in DNA base excision repair (BER), such as OGG1, and found that these levels were decreased in RSTS cells.

Conclusions: We found BER defects in RSTS cells using the Comet and DNA incision assays. Finally, *EP300* mutated cells were transfected with p300-plasmid to complement the diminished protein levels. In the complemented cells the sensitivity to potassium bromate was restored to levels approaching those observed in normal LCLs, indicating that DNA repair defects in RSTS cells are associated to p300 or CBP protein haploinsufficiency.

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P12.184D

Fluorescence in situ hybridization as a tool in the diagnosis of soft tissue sarcomas

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Soft tissue sarcomas (STS) are rare solid cancers of mesenchymal cell origin accounting for <1 % of adult cancers and they represent histologically and molecularly heterogeneous group of tumors.The diagnostics of STS is difficult because of heterogeneity and low incidence, but it is necessary to diagnose the highly malignant sarcomas early and accurately. Specific genetic findings as translocations of *SS18, COL1A/PDGFB, EWSR1, DDIT3* genes have been found in 30 % STS, and they can be detected by fluorescence in situ hybridization (FISH).Between January 2014 and January 2017, in our laboratory, 17 formalin-fixed, paraffin-embedded (FFPE) tissues were examined by FISH using probes:Kreatech ON SYT (18q11) Break, ON EWSR1 (22q12) Break, ON CHOP (12q13) Break, ZytoVysion SPEC COL1A1/PDGFB Dual color.A total of 17 cases were evaluated, namely a Ewing sarcoma (7 cases), Synovial sarcoma (8 cases), Liposarcoma (1) and Dermatofibrosarcoma (1).Rearrangements of investigated genes were identified in 11 samples (64,7 %): rearrangement of *SS18* in 3 cases of synovial sarcoma, rearrangement of *EWSR1* in 4 cases of Ewing sarcoma, rearrangement of genes *COL1A1/PDGFB* in one case of dermatofibrosarcoma and rearrangement of gene *DDIT3* in one case of liposarcoma.In 2 cases of suspect Ewing sarcoma with rearrangement of *EWSR1* the final histopathological diagnosis were olfactory neuroblastoma and extraskeletal myxoid chondrosarcoma.6 FISH negative cases (35,3 %) were reported as stromal endometrial carcinoma, malignant peripheral nerve sheath tumor, leiomyosarcoma, adenocarcinoma, sarcomatoid carcinoma.Rearrangements of investigated genes have been identified by FISH in 64,7 % cases, the findings were corresponding with the final diagnosis and with literature.

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P12.185A

Further support for the 4-hit/3-step model of tumorigenesis in *LZTR1*- associated schwannomatosis

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Introduction: Schwannomatosis (OMIM# 615670) is a tumor predisposition syndrome characterized by benign intracranial and spinal nerve root tumors as well as peripheral nerve sheath tumors that rarely undergo malignant transformation. Mosaic neurofibromatosis type 2 (NF2 - OMIM# 101000) and schwannomatosis may have similar clinical manifestations. Treatment, follow-up and genetic counseling are dependent upon correct diagnosis. Case report: This 61-year-old man presented with a spinal schwannoma at age 51 years and was diagnosed with a unilateral vestibular schwannoma at age 60. Additional findings include four café-au-lait spots and multiple non-tender subcutaneous tumors, one verified as a lipoma. His father has non-tender subcutaneous tumors on both forearms that have not been biopsied, family history is otherwise non-contributory.

Methods/Results: Genetic analyses were performed on DNA extracted from blood and tumor tissue. In blood, MLPA and Sanger sequencing of DNA and cDNA for *NF1* and *NF2* was normal and an exome-based panel for schwannomatosis revealed a novel heterozygous variant in *LZTR1* (NM_006767.3) c.2463dup p.(Asp822Argfs*29). In DNA extracted from a single tumor, MLPA identified a heterozygous deletion of chromosome 22q11q12 which included *LZTR1*, *SMARCB1* and *NF2*, and Sanger sequencing detected a hemizygous variant in *NF2* (NM_000268.3) c.222del p.(Trp74Cysfs*49) as well as the constitutional *LZTR1* variant in the hemizygous state. Parental analyses are pending.

Conclusions: Results of tumor and germline analyses in this individual support the 4-hit/3-step model of tumorigenesis in *LZTR1*-associated schwannomatosis. Comprehensive tumor and germline testing may facilitate differentiation of mosaic NF2 and schwannomatosis.

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P12.186B

Expanded sequencing capacity for increased depth and plexy using the Ion Torrent S5 platform to analyze oncogenic markers

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Introduction: Use of Next Generation Sequencing technology for analysis of genetic markers related to specific cancer types is now a common laboratory technique. The sequencing capacity per instrument use is a limiting factor in the application of sequencing for oncogenic analysis. Here we report system improvements to the Ion S5 GeneStudio platform that double the capacity of the number of samples analyzed per sequencing run.

Materials and Methods: The Oncomine Comprehensive Assay v3 (OCAv3) is an assay that targets 161 genes relevant to solid tumors and is compatible with FFPE DNA and RNA. We used this panel as a test case for the newly released Ion 550 chip, which has double the capacity of the 540 chip.

Results: The 550 chip allowed for simultaneous analysis of 32 samples (16 DNA + 16 RNA) in one sequencing run. Greater than 99% concordance was observed between the 550 chip and the 540 chip with respect to recognition of variants in commercially available controls and tissue samples of known truth. We also used a set of control samples to optimize variant calling parameters for this panel, reducing the frequency of false positive variant calls.

Conclusions: Use of the Ion 550 chip for oncogenic analysis by DNA sequencing supports double the capacity of the 540 chip with no decrease in accuracy or quality. Furthermore, the greater sequencing capacity achieved can also be applied to increasing the throughput or detection limit of other assays, such as liquid biopsies.

J. Gioia: A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **J. Veitch:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **E. Lagier:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **C. Parikh:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **K. Atehortua-Khalsa:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific.

P12.187C

Exome sequencing identified potential candidate genes for serrated polyposis syndrome

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Background: Serrated polyposis syndrome (SPS) is a poorly defined colorectal cancer predisposition syndrome characterized by the occurrence of multiple and/or large serrated lesions throughout the colon. To date, only few molecular signatures have been described and the etiology of the syndrome has not been identified in the vast majority of patients.

Methods: To uncover causative germline mutations, exomes of 31 SPS patients have been sequenced (Illumina HiSeq) using leukocyte DNA. The variants were filtered for rare (MAF: biallelic ≤ 1%, heterozygous ≤0.1% according to dbSNP, EVS, and ExAC), truncating, and missense variants (pathogenic in ≥ 2/3 prediction tools). For data analysis and filtering, the GATK software and the Cartagenia Bench Lab NGS Software were applied; functional scores were used to prioritize the variants.

Results: After stringent filtering steps, potentially biallelic variants were found in 60 genes, with seven genes functioning in known cancer-associated pathways such as DNA-repair and *AKT1*-signaling. Heterozygous variants in at least two patients were found in 334 genes. These encompass four regulators of the oncogene induced senescence pathway, three genes involved in DNA repair and two genes known to be causal for other polyposis syndromes. The most interesting finding was a heterozygous *RNF43* splice-site mutation identified in an index patient and his affected daughter.

Conclusions: The data indicate that exome sequencing might identify causative variants for SPS. The current work-up includes testing of relatives to determine the zygosity of assumed biallelic variants, analyzing the segregation with the phenotype, and functional analyses of the most interesting variants.

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P12.188D

Diagnostic value of SHB expression in human prostate cancer tissue and in benign prostatic hyperplasia

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The general diagnosis for metastatic CaP remains poor and locally advanced disease is difficult to treat successfully. Except the PSA, no specific diagnostic test for CaP is currently available. SHB protein takes part in regulation system of apoptosis, angiogenesis and cell cycle. Reduced tumour growth in vivo and increased c-Abl activity in PC3 prostate cancer cells overexpressing SHB was described in mice. In this first-ever study evaluating SHB expression in human prostate, transcription levels of SHB in prostate cancer and benign prostate hyperplasia was compared. Isolation of total RNA from prostate tissue in 127 patients with histologically proved prostate cancer and 55 patients with benign prostate hyperplasia was performed. After qRT-PCR, comparisons of transcriptional levels in prostate cancer and BPH and in prostate cancer groups classified by staging, Gleason score, age and PSA were evaluated. Underexpression of SHB in prostate cancer tissue comparing to benign prostate hyperplasia ($p< 0.001$) and decreased expression in locally advanced disease (T2 v.s. T3-4)

($p = 0.0066$) were detected. There were no differences in comparison of the groups distributed by Gleason score, age and PSA. SHB was underexpressed in prostate cancer tissue compared to BPH and its expression was lower in locally advanced tumours. This data may be used to make the diagnosis of prostate cancer more precise. Research was supported by Diana Lucina, GAUK 200 090 and IG 00064203.

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P12.189A

Improved performance and workflow using Sanger sequencing for low-level somatic mutation detection from low amount of formalin-fixed paraffin-embedded (FFPE) DNA

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Introduction: Sanger sequencing is often used in oncology research applications for molecular profiling of cancers. Applied Biosystems™ Minor Variant Finder (MVF) Software now enables low-level variant detection in Sanger sequencing traces. RAS mutational testing is frequently performed by clinical researchers due to the strong correlation between RAS mutational profiles of colorectal cancers and their anti-epidermal growth factor receptor (EGFR) response.

Materials and Methods: We have improved and simplified the workflow for the extended RAS research assay. The ready-to-use plates are preloaded with primers for all the eight frequently studied hotspot regions of KRAS and NRAS genes thus only the PCR mix and templates need to be added. Minimized hands-on time, extra convenience and flexibility gained by using the SeqStudio Genetic Analyzer which utilizes an all-in-one cartridge and provides simple plate setup with easy-to-use touch-screen interface and real-time/remote data monitoring.

Results: The performance of the assay on the SeqStudio instrument was tested in the laboratory of Dr. Quagliata (University of Basel, Switzerland) on twenty-two FFPE DNA samples derived from colon cancer biopsies. Variants (ranging from 5.25% to 91.1%) were successfully detected even from research samples where less than 1 ng of input DNA per reaction was used. Only limited amount of microdissected FFPE sections were available for 6 research samples where DNA concentrations were below 1 ng (down to 0.094 ng).

Conclusions: RAS mutations down to 5% level can be detected even from less than 1 ng FFPE DNA with simplified workflow, fast turnaround time and low cost per sample.

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P12.190B

A novel method for detection of somatic L1HS retropositional events in tumor cells

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Introduction: Retroelement activity is one of the important causes of the human genome instability. In previous studies the somatic insertions of L1HS retroelements were revealed in neurons and in different types of tumors. Modern approaches for somatic L1HS detection are based on analysis of whole genome sequences or target massive sequencing of fragments adjusted to retroelement's insertions. However, it is still hard to distinguish true somatic retroelement's insertions and various artifacts. Here we describe an advanced artifact-resistant method for the detection of somatic L1HS insertions.

Materials and Methods: UMI-containing adapters were ligated to restriction digested DNA from 5000 seminoma cells and 5000 normal cells of testicle tissues. Tumor and control libraries were prepared by multiplex PCR with

biotinylated L1HS-specific primers covering 3'- and 5'-flanks of full-sized and truncated L1HS insertions. Obtained amplicons were extracted by streptavidin magnetic beads capture. The filtration of possible artifacts was based on statistical models built on known fixed L1HS insertions.

Results: The use of restriction endonuclease instead of random fragmentation enables to distinguish true flanks from chimeras. Sequencing of captured amplified copies of L1HS insertions allows to use template DNA for further insertion validation. UMI provide accurate count of cells bearing particular somatic insertions. Sequencing of both flanks enables to identify target site duplications - characteristic features of transpositional events.

Conclusions: The developed method is highly sensitive, resistant to various artifacts and is able to detect somatic retroelement insertions in bulk DNA as well as in single cells. Funds: RSF grant #18-14-00244, RFBR #17-04-01280, #16-04-00779.

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P12.191C

Classification of 15 new *BRCA2* exons 2-9 splicing variants by hybrid minigenes

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The clinical classification of BRCA2 variants of uncertain significance (VUS) poses a challenge in human genetics. Historically, variants have been catalogued from the protein outlook, however, upstream gene-expression mechanisms, such as splicing, could be impaired by changes in the DNA sequence. Disrupted splicing variants could alter the ORF provoking the BRCA2 truncation and be involved in cancer development. Here, we have evaluated the splicing effect of 83 BRCA2 exons 2-9 variants by functional assays using the minigene MGBR2_2-9.

The MGBR2_2-9 was built based on the splicing vector pSAD (Patent-P201231427, CSIC). In silico selected BRCA2 variants, from BIC and UMD databases, were introduced into MGBR2_2-9 and assayed in MCF-7 cells. Transcripts were analyzed by capillary electrophoresis and sequenced.

After the bioinformatic analysis of 302 BRCA2 variants, 83 were functionally tested. Results showed that 53 variants impaired splicing (26 intronic and 27 exonic). Among them, 36 provoked ≥2/3 of aberrant transcripts. According to the ACMG and ENIGMA criteria, our results support the re-classification of 12 variants from VUS to pathogenic (c.67+1G>T, c.67+3A>G, c.426-12del5, c.441A>G, c.451G>A, c.475+3A>T, c.516+4delAA, c.517-1G>A, c.517G>T, c.631+1G>A, c.632+3A>G and c.632-3G>C) and 3 from VUS to likely pathogenic (c.97G>A, c.100G>A, c.476-3C>A).

The reproducibility of this technique was supported by previous studies based on minigenes or/and patient RNA. MGBR2_2-9 is a robust tool which provides RNA data for the clinical interpretation of splicing variants.

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P12.193A

Novel regions with LOH in sporadic renal angiomyolipoma

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Novel regions with LOH in sporadic renal angiomyolipoma

Introduction: Sporadic renal angiomyolipoma (RA) is a common benign neoplasm, which occurs in 13 from 10000 people, predominantly in women. Recent researches show contradictory results of mutational profiling of sporadic RAs. According to COSMIC database, 45% RAs have mutations in *TSC2*, which is involved in PI3k/akt/mTOR pathway. Here we demonstrate two novel regions with LOH in 15q14-q15.1 and 16q21-q22.1, apart from six cases with LOH in 16p13.3 in sporadic RAs (table).

Materials and Methods: 409 tumor related genes were sequenced by NGS in 20 samples of sporadic angiomyolipoma tissues from 19 women and one man. For two samples (#6 and #7) micro-array analysis was performed to validate NGS findings.

Results: We have identified in total eight LOH regions, were six were localized in 16p13.3 encompassing *TSC2* gene, and two were novel, one in 15q14-q15.1 and one in 16q21-q22.1. In samples #6 and #7 micro-array analysis revealed uniparental disomy (UPD) and deletion respectively.

Conclusions: Apart from six LOH regions in 16p13.3, for the first time we demonstrate two novel regions with LOH in sporadic cases of renal angiomyolipoma (15q14-q15.1; 16q21-q21.1). In these two cases, no mutations in *TSC2* or *TSC1* were found, as well as in other 407 tumor related genes that were analyzed.

Table. Regions with LOH in sporadic renal angiomyolipoma identified in this study.

Sample #	Coordinates of LOH regions (ISCN 2016)
4	seq[CRch37/hg19]hmz(15)(q14-q15.1) chr15:g.39884882_40494960hmz
6	arr[CRch37/hg19]hmz(16)(p13.3) chr16:g.(1800000_2700000)x2hmz
7	arr[CRch37/hg19] hmz(16)(p13.3) chr16:g.1925000_2120000del
9	seq[CRch37/hg19]hmz(16)(p13.3-p13.11) chr16:g.2129637_15820863hmz
11	seq[CRch37/hg19] hmz(16)(p13.3) chr16:g.2110608_23646191hmz
12	seq[CRch37/hg19] hmz(16)(p13.3) chr16:g.2105335_2138422hmz
19	seq[CRch37/hg19] hmz(16)(p13.3) chr16:g.2136360_3779115hmz
20	seq[CRch37/hg19]hmz(16)(q21-q22.1) chr16:g.65025718_68857441hmz

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P12.194B
Simple, automated somatic structural variation detection and annotation using Bionano Genome Mapping

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Structural variation (SV) detection is fundamental to understanding cancer. While karyotyping and conventional approaches are robust, they can be manually intensive, and biased towards targeted loci.

Bionano Genomics' Saphyr System generates genome maps and detects large SVs. Bionano's variant annotation workflow can uncover rare and sample-specific mutations. To determine variant frequency in a tumor-normal experimental design, it compares the cancer sample's calls against >600,000 SVs from >150 humans with no reported diseases. To identify somatic mutations, the workflow compares against a control sample, and examines whether the cancer mutations are present in low fraction among the control's molecules. Using this pipeline, whose runtime is only a few hours, we can efficiently focus on several dozen significant SV candidates.

We have applied Bionano's DLS labeling chemistry to multiple samples originating from solid and hematologic tumors. Bionano mapping is able to assemble genomes with unprecedented contiguity and accuracy for simple understanding of somatic structural variation. We compared variants from a human breast mammary duct cell line against a matched lymphoblastoid cell line. We identified 45 translocations events, impacting genes such as *RUNX1*. We discovered known rearrangements such as the t(9;22) in chronic myeloid leukemia (CML). Moreover, we uncovered novel mutations ranging in size from a small 4.2 kbp insertion disrupting an acute lymphoblastic leukemia (ALL) gene *CNOT3* to a large 9.6 Mbp deletion at 17q25. In conclusion, Bionano mapping is a cost effective method to detect a broad range of structural variations in leukemias and solid tumors.

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P12.195C
Variants in genes related to oncogenesis in supercentenarians

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Introduction: Supercentenarians are individuals aged 110 or more, and their worldwide number is estimated at no more than a few hundred individuals. It can be assumed that their genome is free of pathogenic variants related to oncogenesis and/or contains protective variants. Discovery of such variants will be of great significance to elucidating the pathohenetic mechnisms of the diseases of the elderly. We tested this hypothesis by analyzing the genomic data of supercentenarians and a control group.

Materials and Methods: We analyze the publicly available whole-genome sequence database from 17 supercentenarians and 34 controls. We filtered the dataset for nonsynonymous variants in genes associated with oncogenesis. We then compared the frequency in variants defined as pathogenic, protective and variants of unclear clinical significance in a freely accessible, public archive of reports of the relationships among human variations and phenotypes *ClinVar*.

Results: Surprisingly, we found pathological variants in the genomes of supercentenarian as we did in the control group. Generally, there was no statistically significant difference in the amount of surveyed variants between the two groups. The genotype frequencies of the variants examined are in Hardy-Weinberg equilibrium, indicating absence of natural selection aginst these genetic variants.

Discussion: The results of our analysis is in concordance with other recent studies that find similar composition of pathologic variants between the genomes of long-lived individuals and controls. We discuss possible explanations of the observed variant frequencies, and we emphasize the need for further studies to clarify the role of variants of unknown clinical significance.

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P12.196D

Investigation of telomerase activity in ulcerative colitis and colorectal cancer patients

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Introduction: Telomerase, a RNA-dependent DNA polymerase which adds telomeric DNA at the 3' ends of eukaryotic chromosomes.Its activity appear elevated in >80% of human cancers. Colorectal cancer is one of the leading cause of cancer-related mortality in most of the world. In the present study, we evaluated telomerase activity in colorectal cancer, ulserative colitis patients and aimed to investigate whether telomerase activity can be used as a malignant marker in premalign lesions such as ulcerative colitis. Normal tissues adjacent to the lesions of the same patients were used as the control group.

Materials and Methods: Tissue samples were obtained from 28 colorectal cancer and 27 ulserative colitis patients during colonoscopy and surgery.Telomerase activity was measured by quantitative telomeric repeat amplification protocol(TRAP)-eze assay.

Results: When we compared the three groups, difference in telomerase activity was detected between colorectal cancer, ulcerative colitis and normal tissue groups. In binary comparisons of ulcerative colitis patients, there was no significant difference in telomerase activity between the diseased and normal tissues($p = 0.113$). However, there was a statistically significant difference between normal and diseased tissues of colorectal cancer patients($p = 0.044$).

Conclusions: There was no association between ulcerative colitis and telomerase activity and we showed that telomerase activity could not be a malignancy marker for precancerous lesions of colon. Our data suggest that telomerase activity detection in colorectal cancer patients may serve as a diagnostic or prognostic marker for malignancy. Further studies are warranted to confirm the diagnostic significance of telomerase activity by quantitative TRAP-eze assay in colorectal cancer.

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P12.199C

Molecular profiles in medullary thyroid carcinomas

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Introduction: Determination of the specific type of thyroid cancer is crucial for the prognosis and selection of treatment of this malignancy. Medullary thyroid carcinoma (MTC) is a histopathological type derived from parafollicular "C" cells of the thyroid, which synthesizes calcitonin. MTC is more aggressive than papillary or folliculary type, comprises 3-5% of thyroid cancers.

Methods: 12 postoperative tissue collected during thyroidectomy and stored as formalin-fixed paraffin-embedded material was used in the study. Immunohistochemical examination it was also essential. RAS and BRAF mutations were characterized by an enhanced polymerase chain reaction followed by restriction fragment length polymorphism analysis (PCR-RFLP).

Results: In all cases, by immunohistochemical diagnostic methods were positive reactions to calcitonin, CEA antigen and thyroglobulin. Chromogranin A and B were moderately positive, neuronal specific enolase and synaptophisine were positive in 15-20% of the tumor cells. Note that both p53 and Ki67, immunohistochemical markers of nuclear proliferation were weak positive. The KRAS mutation was more frequently found than the HRAS mutation, comprising 19.7 % of the RAS mutations, and the V600E

mutation of BRAF was found in 65.2% MTC samples tested.

Conclusions: In conclusion, our results, together with previous genetic studies on thyroid neoplasms, are consistent with the concept that histopathological patterns could be supported by molecular biomarkers to confirm the prognostic and to improve understanding of thyroid tumorigenesis.

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P12.200D

Diagnosis of Li-Fraumeni Syndrome: Differentiating *TP53* germline mutations from clonal hematopoiesis - Results of the observational AGO-TR1 trial

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Introduction: The Li-Fraumeni cancer predisposition syndrome (LFS1) presents with a variety of tumor types and the *TP53* gene is therefore covered by most diagnostic cancer gene panels. While inherited, heterozygous germline variants usually show a variant fraction (VF) of approximately 50%, *TP53* variants with a VF below 50% were described, suggesting *de novo* somatic mosaic variants.

Methods: Using blood-derived DNA, we screened 523 unselected patients with ovarian cancer (OC) for deleterious variants in cancer predisposition genes, including *TP53*, by hybridization capture-based next-generation sequencing.

Results: Potentially deleterious *TP53* missense variants were identified in blood-derived DNA of 6 patients with OC. The VFs of 3 *TP53* mutations detected in 3 patients were 50%, 49% and 55%, respectively, compatible with VFs usually observed for germline variants. These *TP53* mutations were also present in the corresponding tumor samples. In the remaining 3 patients, 4 *TP53* variants with lower VFs of 34%, 26%, 17% and 7%, respectively, were observed. None of these mutations were detected in the corresponding tumor samples.

Conclusions: Deleterious *TP53* variants identified in blood-derived DNA of patients with OC were not causally related to the patients´ cancer in 3/6 *TP53*-positive cases. The evidence for deleterious *TP53* mutations identified purely in blood-derived DNA but not in the tumor of the patient seeking advice may have severe implications for genetic counselling. Our findings may help to avoid false-positive genetic diagnoses of LFS1.

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P12.201A

The role of Trp53 in chemical-induced lung adenocarcinoma

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Introduction: Lung adenocarcinoma (LADC), the prime cancer killer worldwide, features frequent loss-of-function of tumor protein 53 (*TP53*) that portends disease dissemination and poor survival. However, the exact timing and functional role of *TP53* loss in LADC progression is unknown.

Objectives: To time and functionally characterize *Trp53* mutations in murine LADC induced by chemical constituents of tobacco smoke.

Materials and Methods: *Trp53* was deleted in the lungs of *Trp53*-conditional mice (*Trp53f/f*) via intratracheal adenovirus (Ad)-CRE or intercrosses with lung lineage-restricted CRE-drivers. Autochthonous LADC were triggered by intraperitoneal urethane and were examined after six months. LADC cell lines were derived from LADC of *Wt* and *Trp53*-conditional mice and were recombined by Ad-CRE *in vitro*.

Results: 5/62 LADC (8%) and 6/9 LADC cell lines (67%) displayed *Trp53* loss. *Trp53* deletion in airway cells had no effect on primary tumor development and growth, but selectively functioned as a tumor promoter in LYZ2+ alveolar type II cells. *Trp53* deletion in LADC cells *in vitro* induced marked changes in gene expression, abnormalities in cell cycling, and, astonishingly, marked epithelial-to-

mesenchymal transition (EMT) increasing the metastatic potential of LADC cells.

Conclusions: *Trp53* loss in LADC functions in a lineage-restricted fashion to perturb cellular proliferation, but also to promote EMT and metastasis. Our murine data explain the dismal role of *TP53* loss in human LADC.

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P12.203C

TRIM8 participates to the mitotic spindle organization: implication in human diseases and cancer

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TRIM8 is a Ring-E3 ubiquitin ligase with a role in cancer pathogenesis and central nervous system-human diseases. We recently demonstrated the role of TRIM8 in controlling cell growth in glioma. Perturbing TRIM8 expression in Neural Stem Cells (NSC) we explored TRIM8-interactome by immunoprecipitation and LC-MS/MS. A number of identified TRIM8 potential interactors, 11/50 (22%), are related to mitotic spindle formation and cytoskeleton organization. We validated four members of the kinesin-like protein family by co-immunoprecipitation assays, including KIF11 and KIFC1, both involved in mitosis and, if aberrantly expressed, in monopolar spindle organization. Immunofluorescence assays in HeLa cells showed that TRIM8 co-localizes with centrosomes and midbodies, confirming a possible role of TRIM8 in the mitotic spindle machinery functions. We further demonstrated that TRIM8-silencing induced a significant accumulation of monopolar spindles in HeLa, human Fibroblasts and in glioma U87MG cells. Time-lapse live-cell imaging, western blot, and cytofluorimetric analysis confirmed that TRIM8-silencing caused a slow-down of the mitosis progression showing cells that stall in G2/M phases taking up to 113 minutes to enter telophase, compared to 38 minutes taken by control cells. Additional studies revealed that TRIM8 affects chromosomal stability, with a significant increase of micronuclei and aneuploidy (near- haploid cells) in TRIM8-silencend cells.

Our data confirmed that TRIM8 plays a crucial role in the mitotic process and may contribute to the glioma development and neurological disease onset, through a not yet identified mechanism that can control KIF11 and KIFC1 activity and/or protein level.

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P12.204D

Genetic Susceptibility to Triple-Negative Breast Cancer in Cyprus; an NGS panel-testing approach

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Introduction: Triple Negative breast cancer (TNBC) is a very aggressive form of breast cancer (BC), characterized by lack of expression of the estrogen and progesterone receptors (ER/PR), as well as the human epidermal growth factor receptor 2 (HER2). The aim of this study was to assess the distribution of germline mutations in cancer susceptibility genes in Cypriot TNBC patients that tested negative for the *BRCA1/2* genes.

Materials and Methods: Genomic DNA from 84 TNBC patients was sequenced using the TruSight Cancer panel. We followed GATK guidelines and all variants were verified by Sanger Sequencing. Rare variants of uncertain

significance (VUS) were evaluated by seven pathogenicity prediction algorithms, and those predicted as pathogenic from at least five tools, were selected for further investigation.

Results: Six pathogenic mutations were identified in three BC susceptibility genes; *PALB2* (4), *TP53* (1) and *FANCL* (1). Presumed pathogenic germline variants were identified in *PRF1* and *ERCC2* genes; 2 truncating *PRF1* mutations and 1 missense *ERCC2* mutation. Additionally, a novel frameshift mutation was found in *ERCC5* gene, a promising BC candidate. Predicted damaging VUS were identified in several genes including *ATM* (2), *BRIP1* (2), *PALB2* (2), *CDH1* (1), *STK11* (1) and *TP53* (1) BC susceptibility genes.

Conclusions: These results suggest that pathogenic and presumed pathogenic variants in cancer predisposition genes can be detected by large scale panel testing. A larger sample size and functional studies, and/or co-segregation analysis are needed for further conclusions.

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P12.205A
Preparation of TSTA.tumor specific minicircle DNA vector for targeted expression in cancer cells

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Introduction: Preparation of an ideal vector for efficient, safe and targeted expression of transgene is a perfect goal in cancer gene therapy. One of the best approaches for targeted expression in tumor cells is using tumor specific promoters, although the weakness of these promoters limits their success. Two-step transcriptional activation system (TSTA) system can augment the transgene expression using a strong trans-activation factor such as VP16. In this study a tumor specific minicircle, named TSTA-minicircle was prepared that contained hTERT as a tumor specific promoter. VP16 coding sequence and GAL4 DNA binding domain were also located after the core promoter and upstream of the EGFP gene.

Materials and Methods: For construction of the TSTA-minicircle, two expression cassettes including hTERT/GAL4/VP16 and G5/EGFP were prepared using PCR and restriction cloning. They were cloned into a proper parental plasmid, which was transformed into ZYCY10P3S2T *E. coli* strain to generate TSTA-minicircle. Resulted minicircle was transfected to MDA-MB-231 and SKBR3[1] as human breast cancer cell lines, as well as MCF10A[1] as a normal mammary cell line. At last, the expression of EGFP was assessed using qPCR and flow cytometry.

Results: The TSTA-minicircle with the length of 2934 bp was successfully constructed. The vector showed a robust and persistent EGFP expression in MDA-MB-231 and SKBR3 cells compared to normal mammary MCF10A cells.

Conclusions: Our results showed that combination of the minicircle as a safe vector with TSTA element results in an ideal vector for more safe and robust expression of the transgene specifically in cancer cells.

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P12.206B
Genetics and expression profile of tubulin gene superfamily in breast cancer

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Introduction: Taxanes are a class of chemotherapeutic agents that inhibit cell division by disrupting mitotic spindle through the stabilization of microtubule. Most of breast cancers (BC) tumors show resistance against taxanes partially due to alterations in tubulin genes. In this study, using genomics databases, we analyze genetic alteration, mRNA expression and the activity of the cis-regulatory elements of 28 tubulin genes in BC subtypes and taxane-resistant BCs.

Materials and Methods: Whole exome sequencing, and expression array profiles were obtained from cBioPortal and Gene Expression Omnibus (GEO) databases. Cistrome Dataset Browser was used to analyze H3K4me1 and H3K4me3 mark enrichment. Data from 529 breast tumors, 8 normal breast biopsies and 5 BC cell lines were analyzed.

Results: Comparative expression levels of tubulin genes were determined in: BC versus normal breast biopsies, PAM50 luminal A, B, HER2-enriched and basal-like BC tumors and cell lines, taxane-resistant versus taxane-sensitive BC tumors and cell line, and the tumors with

pathologic complete response (pCR) to taxane treatment compared to non-pCR. We found that mRNA down-regulation and gene amplification were the most frequent alteration in the tubulin genes. Frequency of mutations in each tubulin gene was analyzed in the four BC subtypes. Enrichment of H3K4me1 and H3K4me3 marks were studied for the all tubulin genes in different BC cell lines.

Conclusions: Different genetic and expression profile of tubulin genes were found in the four subtypes of BCs and in the taxane-sensitive and the -resistant BC. This study was supported by Women and Children's Health Research Institute (WCHRI).

B. Nami: None. **Z. Wang:** None.

P12.208D

A rare case of hereditary uveal melanoma as part of Li-Fraumeni-like syndrome

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Introduction: Uveal melanoma (UM) is the most common primary malignant tumour of the uveal tract with distinct molecular-genetic features that allow to distinguish UM from other subtypes of melanoma. Somatic mutations in UM affect some genes - *BAP1, GNA11, GNAQ* et al., that determine the biology and behaviour of a tumour and appear to be the predictors of disease. In about 2-5% of cases the manifestation of UM is associated with a hereditary condition and could be a result of germline mutations in the genes responsible for some syndromes. Currently several syndromes have been described such as *BAP1*-associated, FAMMM, Li-Fraumeni and others.

Materials and Methods: We present here the case of a 62-year-old woman with Li-Fraumeni-like syndrome (LFL) who had late UM of the left eye. Pedigree analysis identified 5 first-degree and second-degree relatives with different tumours including a 73-year-old sister who had been previously treated from melanoma of horioidea and a 52-year-old brother suffered from renal cancer. Given the extensive family history of late-onset malignancies, the sample of peripheral blood lymphocytes of the patient was assessed for *BRCA2* and *CHEK2* mutation using Real-time PCR followed by Sanger sequencing.

Results: The analysis revealed a germline heterozygotic *CHEK2* mutation (c.470T/C, p.Ile157Thr, rs17879961). The patient's sister underwent genetic screening as well and was found to carry the same mutation.

Conclusions: We suggest LFL for the family with late-onset cancer and low penetrance. The surveillance program was adjusted to the elevated risk for other primary malignancies both for the patient and her siblings.

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P12.209A

VISIon: Von Hippel-Lindau Information Technology-Sharing International Consortium

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Introduction: Von Hippel-Lindau (VHL) disease is an inherited cancer predisposition condition with diverse clinical manifestations spanning multiple organ systems. Germline variants in the VHL gene identify most VHL families. Certain VHL variants have been shown to predispose individuals to distinct manifestations. To collate the genetic impact of variants in VHL, we initiated an international data-sharing consortium to develop a standardized genomic and phenotypic data capturing protocol.

Methods: Our initial data set comprises 2251 cases from existing cohorts in the Netherlands and Canada, as well as published literature and publicly-accessible databases. We have developed a standardized data collection protocol that captures all aspects of the VHL phenotype to collect genotypic and phenotypic data from the collaborating sites. We have worked closely with the National Institutes of Health Clinical Genome Resource to define a platform to share our data. We will store the data in CIVic (Clinical Interpretation of Variants in Cancer), an open source database for genotype-phenotype correlations, and then contribute this data to the NIH's ClinVar, a freely-available public datasharing resource that facilitates collaboration with scientists in VHL.

Conclusions: By collating VHL patients and mutations, we plan to create the most comprehensive VHL data-sharing mechanism to best understand VHL. We hope to define new patterns of phenotypic expressions of known mutations. We invite collaborators with VHL databases or cohorts to contribute cases to improve the characterization of genotype-phenotype relationships in this complex disorder, and will make the data collection tools freely

available to interested investigators. Source of Funding: VHL Alliance.

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P12.211C

Detection of copy number aberrations and loss of heterozygosity in wilms tumour DNA samples from formalin-fixed paraffin-embedded tissue using molecular inversion probe-based array

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Introduction: Wilms tumour (WT) is the most common renal tumour in children. Loss of heterozygosity (LOH) for chromosomes 1p and 16q is an adverse prognostic factor in WT. The aim of this study is to characterize WTs using Molecular Inversion Probe-based (MIP) Array to identify copy number aberrations (CNAs) and LOH.

Materials and Methods: Genomic DNA were extracted from 8 formalin-fixed paraffin-embedded (FFPE) sections of WTs using Promega Maxwell RSC Instrument and purified as input into Affymetrix OncoScan FFPE Assay Kit. Data was analysed with Chromosome Analysis Suite software version 3.2.0.1252.

Results: We saw gains of chromosomes 1q and 7q; segmental gains of 1q21.1-q32.1, 2p24.3, 4q31.3, 11p14.3, 14q11.2, 15q21.3, 16q22.2, 17q24.1, 17p11.2-q25.3, 19p13.11, 22q13.33 and Xp22.33; losses of 1p, 7p, 10p, 11 and 14; and segmental losses of 3p25.2, 4q12-q13.1, 4q13.3-q35.2, 8q24.3, 10p11.23, 11p15.4, 16p13.3, 16q22.2, 19q13.2, 19p13.2, 19p13.3, 22q12.3-13.33, 22q11.23-q12.1 and Xq11.1-q11.2. The most common aberrations were gains of chromosomes 12(5/8), 13(4/8), 7(3/8), 7q(2/8) and 8(3/8); and loss of 7p(2/8). Frequent LOH seen were 1p36.11-p35.3(2/8), 1p31.1(2/8), 1p33-p32.3(3/8), 1q21.2-q21.3(3/8), 3p21.31-p21.31(6/8), 3p12.2p12.1(3/8), 6p22.1(2/8), 7q11.1-q11.21(3/8), 10q11.21-q11.23(2/8), 11p(3/8), 12q21.3-q21.33(3/8), 17p11.2-p11.1(5/8) and 17q23.1-q24.1(2/8).

Conclusions: Of 8 WTs, we identified LOH at 11p in 3 cases, a frequency consistent with reported literature, and found LOH in 1p and 16q(1/8). Our data also supports published reports that trisomies 6, 7, 8, 12, 13 and 18 are the most common aneuploidies in WT. Our findings demonstrate the potential of MIP-based assay towards the identification and mapping of important CNAs and LOH involved in WT for its prognostication.

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P12.212D

Using archived FFPE samples for retrospective miRNA expression profiling of blastemal Wilms' tumors by qRT-PCR

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Introduction: Wilms' tumor is the most frequent genitourinary malignancy in young children. A high percentage of blastema after pre-operative chemotherapy (according to the SIOP protocol) is a sign of poor prognosis. Differences between miRNA signatures of blastemal and regressive tumor subtypes may hold relevant information on genetic factors underlying chemo-responsiveness.

Materials and Methods: We extracted miRNA from two FFPE samples per patient: a tumor sample and a tumor-free region of the same kidney to be used as control. A PCR array was used to reveal miRNAs of interest in the first patient and the expression of selected miRNAs was studied in seven other patients by individual qRT-PCR primers.

Results: MiRNA expression patterns obtained from FFPE samples showed a close resemblance to the results of previous profiling reports using fresh-frozen tissue. Relevant differences include miR-184 found to be more underexpressed than expected, and miR-203a, which we report to be downregulated in Wilms' tumor for the first time to our knowledge.

Conclusions: Despite favorable reviews in the literature with respect to various diseases, miRNA expression analyses from FFPE samples are still rarely reported. We hereby demonstrate the usefulness of pathological archives in Wilms' tumor profiling. Putting our results in perspective with literature data, miR-184 seems to be underexpressed in a subset of blastemal and possibly other Wilms' tumors. Downregulation of miR-203a may or may not be specific to blastemal tissue, but it could explain the overexpression of E2F3 thought to be important in the pathogenesis of the disease.

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P12.213A

Global binding pattern of mutant WT1(R394W) to the genome in leukemic cells

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Introduction: Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults. Mutations in Wilms' tumor gene 1 (WT1) are present in 10-15% of cytogenetically normal AML and are reported as independent negative prognostic factors. The recurrent WT1 (R394W) mutation changes the basic arginine to an uncharged tryptophane. Since Arg^{394} contacts DNA, the binding of WT1(R394W) may be adversely affected. The aim of this project was to characterize the binding pattern of WT1(R394W) as compared to the binding of the wild type WT1-KTS and WT1+KTS isoforms.

Material and Methods: From leukemic K562 cells expressing BIO-tagged WT1(R394W), we performed chromatin precipitation by streptavidin capture, followed by deep sequencing.

Results: Three independent experiments yielded 470 overlapping peaks. We found that the R394W mutation in a WT1-KTS background deprived WT1-KTS of its binding close to transcription start sites of target genes. WT1 (R394W) shows a reduced binding affinity and, similar to WT1+KTS, binds mainly within the gene bodies of target genes. Upon transient overexpression in HEK293 cells, only WT1-KTS showed evidence of transcriptional activation of target genes common to WT1(R394W) and the wild type -KTS and +KTS isoforms. While overexpression of WT1-KTS in K562 cells conferred resistance to the tyrosine kinase inhibitor Imatinib, overexpression of *WT1*R394W conferred only partial resistance.

Conclusions: Taken together, our results support the notion of *WT1*(R394W) as a loss of function mutation. The functional consequences of the acquisition of binding to the gene body within target genes, in a manner similar to WT1 +KTS, remain to be clarified.

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P12.214B

Modeling human hereditary cancer syndromes using CRISPR/Cas9 mediated genome editing in *Xenopus tropicalis*

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CRISPR/Cas9 and TALEN mediated genome editing creates unique and unmatched opportunities for modeling human disease in non-mammalian model organisms. The amphibian *Xenopus tropicalis* is extremely well positioned for this approach. It shares with zebrafish the aquatic habitat and easy manipulations associated with its external development. However, it manifests unique features making it a powerful organism for modeling human genetic disease. (1) unlike zebrafish, *Xenopus tropicalis* has a true diploid genome, precluding redundancy. (2) Its genome shows high synteny with humans, greatly facilitating identification of disease orthologs. (3) Gene manipulations can be restricted to specific tissues and organs via targeted blastomere injection. We have recently generated the first genetic cancer models in *Xenopus tropicalis*. Via mosaic targeting of the tumor suppressor gene *apc* we generated tadpoles that rapidly (< 1.5 months) and efficiently (>90%) developed a range of neoplasia characteristic for Familial Adenomatous Polyposis (Van Nieuwenhuysen *et al*., Oncoscience 2015). Similarly, we found that *rb1/rbl1* double mosaic mutant tadpoles rapidly develop retinoblastoma (Naert *et al*. Sci. Rep. 2016). More recent work also indicates the possibility of modeling Li-Fraumeni, T-cell acute lymphoblastic leukemia and pancreatic neuroendocrine cancer. The rapid kinetics of tumor development in *Xenopus* pave the way for their use as pre-clinical model, providing unique possibilities for fast identification of modifier genes and novel drug targets. We present our first promising results with multiplexed gene targeting in desmoid tumors. We believe that these models offer a unique experimental platform to contribute to the field of human cancer and medical genetics.

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P12.215C

Young breast cancer: Predisposed to aggression?

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Introduction: 2% of breast cancers occur in women ≤35 years of age. Limited data suggests that up to 15% may be hereditary, a high proportion are aggressive (triple-negative, TN or HER2+), and frequently diagnosed at an advanced

stage. Canadian-specific data of this population is lacking and would serve to improve risk assessment and genetic counselling.

Methods: A retrospective chart review examined the characteristics of women diagnosed with breast cancer ≤35 years and who received treatment/genetic counselling at Princess Margaret Cancer Centre (PM) in Toronto, Canada after 2000.

Results: Of 226 women identified, 207 received testing at PM. Overall rate of pathogenic variants was 19% for *BRCA1/2* and 7% for other genes. Most women had stage 2 disease (42% overall); however, 33% of carriers compared to 24% of non-carriers were ≥ stage 3. Most tumors were ER+/PR+/HER2- (45% overall); however, 61% of carriers presented with aggressive disease (45% TN, 16% HER2+) as compared to only 46% of non-carriers (21% TN, 25% HER2+). Of note, 86% of *BRCA1* were TN, 75% of *BRCA2* were ER+/PR+, and 60% of *TP53* were HER2+. Additionally, 22% of carriers reported no family history of breast/ovarian cancer.

Conclusions: This study demonstrates that early-onset breast cancer has distinct features that vary based on genetic status. Overall, carriers tend to present with later stage and more aggressive disease as compared to non-carriers. Given the high mutation rate, and that many carriers report no family history, it is important that all women with this diagnosis be offered genetic testing.

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P12.216D
Neurofibromatosis and Related Tumors: molecular markers associated to high risk for tumor-development

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Introduction: Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder, caused by inactivating mutations of neurofibromin gene (17q11.2). Nf1 gene encodes for five different isoform. Few cases of genotype-phenotype have been reported. Several studies showed that alterations in the type I versus type II mRNA ratio of nf1 gene can be associated with the presence of malignancies including colon cancer, neuroectodermal tumors and ovarian cancer in patients not affected by NF1.

Materials and Methods: The study was conducted on a cohort of 144 NF1 patients, age 1-57 years, and 144 age and sex matched controls. The patients were divided into three groups (mild, moderate and severe phenotype). The mRNA levels of isoform 1 and 2 of the nf1 gene in peripheral blood leukocytes of NF1 patients and of controls was assessed by real-time PCR. Statistical analysis was performed using t-test.

Results: Molecular analysis showed a wide distribution of each mutation along the NF1 gene. Expression levels of isoform 1 were lower in patients than in controls (0,00066 vs 0,0012, p = 0,0000055), and the patients with severe phenotype showed levels lower than mild patients (0,0005 vs 0,00074, p = 0,02). Expression levels of isoform 2 were lower in patients than in controls (0,0024 vs 0,01, p = 0,0004).

Conclusions: The identification of an association between specific NF1 expression pattern and severity of phenotype might help to establish a specific prognosis and consequently to start a specific follow-up program and eventually a specific therapeutic approach in NF1 patients.

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P13 Basic mechanisms in molecular and cytogenetics

P13.01A
Comparison between functional predictors and in vitro effect of alpha-1 antitrypsin missense variants

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Introduction: The alpha-1 antitrypsin (AAT) coding gene SERPINA1 is highly polymorphic with more than one hundred variants described in databases. Although the functional implications of the most common mutations S and Z have been well characterized, the effect of many newly identified variants has not been empirically

demonstrated, and it is unclear whether bionformatic softwares are able to correctly predict their pathogenicity.

Materials and Methods: We have analyzed the coding sequence of SERPINA1 gene in patients with AAT deficiency (AATD) and have identified 12 previously undescribed missense variants. Different algorithms were used to predict the effects of these substitutions. In addition, mutant proteins were expressed in vitro and functional assays were performed to empirically determine the pathogenicity of the variants (western blot analysis, PAS staining, elastase inhibitory assay, pulse-chase experiments).

Results: Most of the variants had a functional impact when overexpressed in a cellular model: intracellular polymerization, impaired secretion, intracellular stabilization and/or reduced anti-elastase activity. Only in one case no pathogenic effect was identified. For most of the variants the functional assay was consistent with the bioinformatic prediction, although in some cases discrepancies between algorithms were observed and for one variant the predictors failed to identify the pathogenic effect of the amino acid substitution.

Conclusions: Functional studies are essential to unravel the molecular mechanisms affected by newly identified genetic variants. Although helpful, functional prediction algorithms are not always correct in their predictions.

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P13.02B

Patient-specific variants identified by whole-exome sequencing underlie discordant phenotypes in familial apparently balanced translocations

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Familial apparently balanced translocations (ABTs) segregating with discordant phenotypes are extremely challenging for interpretation and counseling. We report four families, each including individuals with identical ABTs and discordant phenotypes. All mechanisms underlying differential phenotypes were thoroughly investigated using FISH, array-CGH, and whole-genome mate-pair sequencing; however, no associations were determined, therefore whole-exome sequencing (WES) was performed.

In this study, the same families were revisited using WES. Disease-candidate variants were validated with Sanger sequencing and their pathogenic impact was assessed with *in silico* tools.

In family 1, WES revealed a novel, patient-specific heterozygous splice donor variant in *STXBP1* (NM_003165.3: c.1110+2T>G), which is essential for neurotransmitter release through syntaxin regulation. Similar patients with epilepsy and intellectual disability have been reported carrying heterozygous *STXBP1* disruptions. The variant identified is predicted to disrupt normal *STXBP1* splicing. In family 2, WES identified a novel, patient-specific heterozygous missense variant in *TUBA1A* (NM_006009.3: c.875C>T), one of the main microtubule components with important roles in neuronal migration. Similar patients with intellectual disability, microcephaly, and lissencephaly have been reported carrying heterozygous *TUBA1A* variants. In families 3 and 4, a novel, patient-specific heterozygous missense variant in *SCN1A*, and a compound heterozygous variant in *C5orf42* were identified, respectively; however, further investigation is required to determine pathogenicity.

In conclusion this study supports our previous publication demonstrating that in the majority of familial ABTs with discordant phenotypes, translocations are coincidental to phenotype, unlike *de novo* ABTs. Instead, patient-specific variants identified by WES seem to underlie phenotype associations in these families.

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P13.03C

Influence of human X chromosome structural variations and aberrations on X chromosome inactivation

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X chromosome inactivation (XCI) balances the expression of X-linked genes between females and males. Early in female development, cells transcriptionally silence one randomly chosen X chromosome. This leads to a general ratio of 50:50 cells, where half the cells inactivate the maternal and half the paternal X chromosome. The ratio varies among women, although significant deviations from it are relatively rare among phenotypically normal women and are known as skewed XCI. Skewing is more common among older women or women with certain X-linked diseases or X chromosome aberrations. The latter is often marked by inactivation of the defective X chromosome. Our study focuses on detecting the influence of X chromosome deletions and duplications on XCI. Two groups of women were selected from the Estonian Genome Center at the University of Tartu population-based biobank, genotyped with high-density whole-genome SNP BeadArrays. PennCNV programme was used for CNV calling according to the manufacturer's protocol. Subjects group was comprised of 436 women with X chromosome aberrations over 50 kb in size spanning the whole X chromosome, while the controls group was comprised of 436 women without X chromosome defects of the mentioned size. Both sample sets were further divided into age groups, taking into consideration the increased rate of skewing in older women. XCI ratio was assessed using HUMARA method. No significant difference between XCI ratios of women with and without X chromosome aberrations has been found so far. However, as expected, an increase in XCI skewing is seen in older women.

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P13.04D

Defective trafficking of inflammatory response factors exhibits hyposensitive immunogenic response in skin fibroblasts from Ataxia Telangiectasia patients

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Background: Ataxia-Telangiectasia (A-T) is a rare autosomal recessive disease affecting cerebellum, immune system, lungs, liver and characterised by an enhanced tumor risk. Despite the cerebellar degeneration, the major cause of mortality in A-T is due to respiratory failure caused by recurrent bacterial infections of the upper-respiratory tract and oral tissues .Since inflammation is emerging as an important hallmark in A-T, we hypothesized that A-T patients could exhibit impaired innate immune response due to defective pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs) by microbes.

Methods: Primary skin fibroblasts from A-T and healthy controls and Hela cell lines

ATM $^{-/-}$ were assessed for genes encoding inflammatory response factors using RT-qPCR and citofluorimetric analysis, before and after stimulation with E.Coli lipopolysaccharide (LPS). LPS and TNF-α mediated Nf-kβ activation was measured by western blot analysis.

Results: Cells lacking ATM protein were less responsive than controls as shown by the defective gene expression of TLR-4 and IL-6 and a significant reduction of IL-6 secreted protein both at basal level and after LPS or TNF-α stimuli. In the same conditions, the

Nf-kβ activation pathway was less activated in A-T cells respect to healthy LCLs.

Conclusions: Our studies indicate for the firt time a defective trafficking of TLR-4 in response to LPS stimuli. This defect could contribute to hyposensitive response of A-T patients to immunogenic challenge. Further investigations in this pathways coul provide a potential target for therapeutic clinical intervention in A-T.

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P13.05A

Breakpoint mapping at nucleotide resolution in balanced translocations associated with clinical phenotypes

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Precise breakpoint mapping of balanced chromosomal rearrangements is crucial to identify disease genes. We evaluated 11 female patients with balanced reciprocal translocations associated with phenotypic alterations. We mapped and sequenced their breakpoints, assessed the rearrangements' impacts on expression of disrupted genes, addressed candidate genes to position effect, and inferred mechanisms of formation. Four out of 11 patients presented one of the chromosomal breaks in heterochromatic and highly repetitive DNA segments, such as centromeres or short arm of acrocentric chromosomes. We demonstrated that nucleotide resolution characterization of breakpoints at gaps in the reference genome is feasible when cytogenomic methods and short-read sequencing are associated. Most of the rearrangements were possibly formed by non-homologous end joining with breakpoints within repeat elements. Seven of the 11 patients presented with breakpoints within a total of nine genes. Seven of which showed altered expression levels and the functional impairment of two of them, e.g. *KIAA2022*, *IL1RAPL1*, could be considered causative of the patients' phenotypes. The disruption of a promoter region triggered the description of a novel X-linked syndrome caused by *AMMECR1* loss of function. In four patients, there was no gene disruption at the breakpoints, suggesting other pathogenic mechanisms. Four candidate genes were considered potentially affected by position effect and expression abrogation of one of them, e.g. *TSPAN7*, was confirmed. We emphasize the importance of breakpoint-junction characterization at nucleotide resolution in balanced chromosomal rearrangements to reveal the genetic mechanisms associated to the patients' phenotypes, mechanisms of formation, and genomic nature of the disrupted DNA sequences.

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P13.06B

Functional analysis of a novel gene X causing *small eye* phenotype through JNK-dependent apoptosis pathway

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Introduction: A bioinformatics work comprising co-expression gene network in the mouse was built based on microarray and RNA-Seq platforms, revealed an unexplored gene *X* which might play a role in retinal development. *X* contains an evolutionary conserved protein domain with a function of oxidative stress resistance by scavenging reactive oxygen species (ROS), but the gene function remains to be explored. The aim of this study was to elucidate *X* gene function, mechanism and its role in eye development using *Drosophila* and mice as a model organism.

Materials and Methods: The GAL4-UAS system were used to specifically knockdown homologue of *X* in the eye of *Drosophila* and resulted in the small eye phenotype. Various genetic crosses were performed to analyze the phenotype were mediated through apoptotic or autophagy pathway. The mice homologous gene knockout was generated using CRISPR/Cas9 technology.

Results: Knockdown of *X* in *Drosophila* resulted in severe small eye phenotype, and the knockout mice were blind. In *Drosophila*, P35 baculovirus overexpression (active caspase inhibitor) and knockdown of pro-apoptotic genes showed complete rescue of small eye phenotype. To further identify the exact apoptotic pathway, various players involved in JUN N-terminal kinase (JNK) apoptosis pathway rescue the small eye phenotype.

Conclusions: The small eye phenotype of *X* could be involved in the JNK-mediated apoptosis pathway. Our future studies would be to prove JNK dependent apoptosis, the role of ROS, mutagenesis study to explore more about the function of *X*.

D. Suresh: None. **Y. Ching:** None. **M. Lin:** None. **B. Liao:** None.

P13.07C

Whole Genome Sequencing of 9 patients allowed a better understanding of complex chromosomal rearrangements

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Chromosomal rearrangements are used to be considered as complex when involving at least 3 breakpoints on two different chromosomes. Cytogenetic microarrays and whole genome sequencing (WGS) revealed rare much more complex situations with numerous breakpoints on a single chromosome overshooting the first definition. Henceforth grouped under the ***chromoanagenesis*** term mechanisms generating such rearrangements remain misunderstood and their definition elusive especially since such constitutional complex chromosome rearrangement (CCR) are rare.

We performed WGS for 9 probable constitutional chromoanagenesis: 4 cases with a minimum of 4 Copy Number Variations on a single chromosome and 5 cases with at least 10 chromosome breakpoints identified in patients with balanced chromosomal rearrangements characterized with WGS (ANI project). We analyzed paired-end WGS data using BreakDancer and ERDS respectively for breakpoint and CNV calling. Our Svagga pipeline was used for filtering and annotation.

All rearrangements appeared to be more complex than initially thought with a total of 232 breakpoints and a maximum of 74 breakpoints clustered in 4 hotspots for a single patient. No statistical significant imbalance was observed compared to random distribution regarding TAD disruption or purine/pyrimidine nucleotide at breakpoint. A statistical depletion of gene-disrupting breakpoints was observed compared to theoretical distribution ($p = 0{,}0016$). Nucleotide resolution showed the combination of several repairing mechanisms within a rearrangement adding complexity to complexity.

Gathering several exceptional observations we help to delineate the chromoanagenesis phenomenon. Breakpoint distribution compared to simpler rearrangements will help understand its origins and provide new insights in **cytogenomics** such as guidelines for structural variant pathogenicity classification.

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P13.08D

Contribution of CMA to genetic diagnosis of individuals with dysmorphisms: a collaborative study of the SIGU (Italian Society of Human Genetics) Cytogenetic and Cytogenomic working group

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Chromosomal microarray analysis (CMA) significantly increased the possibility to identify copy number variations (CNVs) associated with a wide range of genomic disorders. Here we report on a collaborative study of the SIGU(Italian Society of Human Genetics) Cytogenetic and Cytogenomic working group, on 780 patients referred to CMA for dysmorphisms as the only clinical manifestation(21%) or associated with intellectual disability/developmental delay (57%), congenital malformation(s)(10%), autism spectrum disorders(6%), epilepsy(3%) or growth anomalies(3%). Overall 329 non-polymorphic CNVs have been identified in 266 patients(34%) of which 78 CNVs have been detected in 56 patients(21%) with dysmorphisms as the only clinical manifestation. In 36% of these probands the CNVs have been classified as pathogenic(pCNVs), with 15% associated to known syndromes, and in 64% as variants of uncertain clinical significance(VOUS). The remaining 251 CNVs have been detected in 210 patients referred for dysmorphisms combined to another clinical manifestation and classified as pCNVs in 38% of cases, with 21% associated to known syndromes, and 62% as VOUS. The average size was 2,9 Mb for all CNVs, >6 Mb for pCNVs and <1 Mb for VOUS. This study allowed us to assess the detection rate of CMA for patients referred for dysmorphisms as the only clinical manifestation, hence informing about the consistent or possible underlying genetic causes to validate/explore in the distinct patients groups by 3d-facial-analysis. Dysmorphisms combined to other clinical findings could be evaluated in the same study and carriers of pCNVs received their diagnosis, while those with VOUS remain amenable to be solved by novel literature insights.

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P13.09A

Allelic gene expression profiles revealed by homologue specific exome sequencing

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Chromosome translocations can be detected by cytogenetic analysis, but this is independent of expression data which do not identify the origin of alleles. It is known that the number of abnormal chromosomes in tumor cells is generally increased during progression in vivo or serial passage in vitro due to chromosome instability. This raises a fundamental question about whether chromosome rearrangements can cause effects on gene expression in rearranged chromosomes in addition to fusion genes. Chromosome sorting by flow cytometry produces flow karyotypes that enable the distinction between normal and abnormal chromosomes. In this study, a derivative chromosome t(9;14) and its homologous normal chromosomes 9 from the Ishikawa 3-H-12 cell line were sorted to collect homologue-specific samples. Chromosome sequencing of the der(9) identified the breakpoint junction at 9p24.3 and 14q13.1 and uncovered the formation of a fusion gene, WASH1-NPAS3. Of 293,903 amplicons in the Ion Ampliseq exome panel, 11,809 amplicons are localized in chromosome 9, corresponding to 749 genes. Chromosome-specific exome sequencing of sorted chromosomes demonstrated that 87% of the chromosome 9 exome was amplified in der(9), which include 982 SNVs. This permits the assignment of allelic variants and can lead to comparisons between normal and abnormal chromosomes. Compared to the RNA sequencing data with the allele-specific variant profiles, each gene expression along chromosome 9 is assigned to both or one of alleles. We show that allele-specific chromosome sequencing of homologues is a robust technique for distinguishing alleles and this provides a valuable approach for the investigation of chromosome instability.

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P13.10B

Very short DNA segments can be detected and handled by the repair machinery during germline chromothriptic chromosome reassembly

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Detailed analyses down to the nucleotide resolution reveal unexpected complexity of seemingly simpler and balanced chromosomal rearrangements. This concerns also chromothripsis, a rare type of complex rearrangement involving local shattering of one or more chromosomes and random reassembly of the resulting segments. Chromothripsis can influence expression of many genes and cause abnormal phenotypes. We studied the structure and mechanism of a seemingly balanced *de novo* chromosome rearrangement in a boy with developmental and growth delay. Karyotyping and mFISH identified 11 segments from four chromosomes to participate in the rearrangement. Microarray analysis revealed two *de novo* deletions of 0.7 and 2.5 Mb at two of the breakpoints in 1q24.3 and 6q24.1-q24.2, respectively. They affected paternal chromosomes and possibly explained most symptoms of the patient. Subsequent whole-genome mate-pair sequencing revealed that the four chromosomes were in fact broken into 29 segments longer than one kb. Sanger sequencing of all junctions showed additional complexity compatible with the involvement of different repair pathways. A translocation of a 33 bp long fragment to one of the junctions was observed which may have implications for the definition of the lower size limit of structural variants. Our observations and review of published chromothripsis events indicate that even very small fragments from the shattered chromosomes can be detected and handled by the repair machinery during germline chromothriptic chromosome reassembly. Supported by 17-29423A, 00064203, 00064165, LM2015091 (Czech Ministries of Health and Education), 2013-14290 (Lundbeck Foundation), Global Genes, Local Concerns (University of Copenhagen) and 4183-00482B (Danish Council for Independent Research).

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P13.11C
New evidence of chromothripsis in congenital disorder

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Introduction: Chromothripsis is a one-step genome-shattering catastrophe resulting from disruption of one or few chromosomes in multiple fragments and consequent random rejoining and repair leading to complex chromosomal rearrangements. While chromotripsis has been extensively observed in cancers, few investigations documented a similar phenomenon in congenital disorders. We report a case of a newborn, conceived using a donor egg, with a complex karyotype and phenotypic abnormalities including congenital cardiomyopathy, genital ambiguity, agenesis of corpus callosum, bilateral ocular abnormalities and absence of left 12th rib.

Methods: Conventional cytogenetic analysis, array comparitive genomic hybridization, whole genome sequencing (WGS), and polymerase chain reaction were used to identify the chromosome rearrangement and characterize the breakpoints in our patient and his father.

Results: Conventional and molecular cytogenetic analysis showed a complex karyotype with structural variations including deletions of three regions on chromosome 2, deletions of two regions on chromosome 4 with inversion of the fragment between the two breakpoints, and a pericentric inversion of chromosome 11. WGS analysis detected further multiple complex balanced intra-chromosomal rearrangements of chromosomes 2 and 4, with 17 breakpoints resulting in the breakage of 11 genes. Paternal origin of the abnormal chromosomes has been confirmed.

Conclusions: The pattern of random joining of chromosomal fragments observed in our case suggests that a chromothripsis event might have driven the formation of these complex rearrangements. The rearranged chromosomes were demonstrated to be of paternal origin suggesting that a chromothripsis occurred during spermatagenesis although the onset in the preimplantation embryo cannot be excluded.

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P13.12D
Medical consequences of pathogenic CNVs in adults

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Background: Copy number variants (CNVs) increase risk for learning difficulties and early-onset neurodevelopmental

disorders but their role in medical outcomes in middle- and old age is limited. The UK Biobank, with half a million well phenotyped adults, presents an opportunity to study the medical consequences of CNV in the general population.

Methods: We analysed 54 pathogenic CNVs in all Biobank participants. We used logistic regression analysis to test CNVs for associations with 58 broad medical phenotypes, present in at least 2000 participants.

Results: CNV carriers had an increased risk to develop 37 of the 58 phenotypes at nominal levels of statistical significance, with 19 of these associations surviving Bonferroni correction for 58 tests. Individual comparisons of each of the 54 CNVs against the 58 phenotypes produced 18 associations that survived Bonferroni correction for 3132 tests and a further 57 that were significant at a false discovery rate of 0.1. Thirteen CNV loci had three or more significant associations at FDR=0.1, with 16p11.2 deletions leading the list with 15 significant results. The most common CNVs (at 0.5-0.7% frequency) have none or minimal impact on medical outcomes in adults.

Conclusions: Some of the 54 CNVs proposed to be pathogenic have profound effects on physical health, even in people who have largely escaped early neurodevelopmental outcomes. Our work provides clinicians with a morbidity map of potential outcomes among carriers of these CNVs, which will be made available on a dedicated web resource, updated as new data is released by the Biobank.

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P13.13A

Congenital adrenal hyperplasia in paediatric age<:> molecular analysis of the *CYP21A2* gene and implications for genetic counselling

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Introduction: Congenital adrenal hyperplasia(CAH) is due to 21-hidroxilase deficiency(21-OHD) in about 95% of the cases. 21-OH is encoded by *CYP21A2* gene, and most frequent mutations occurring in *CYP21A2* are due to gene conversions originated from its pseudogene(*CYP21A1P*). The clinical severity of CAH is associated with the impairment of 21-OH activity, which is directly related with the molecular defect. CAH is classified as classic salt-wasting(SW) and simple virilising(SV) forms, and non-classic(NC) form of the disease. SW and SV are usually diagnosed after birth or during the first years of life, respectively, while most cases of NC-CAH are diagnosed during infancy, puberty or until adult age. Here we present the molecular results performed in paediatric patients with CAH.

Methodology: molecular analysis (using genomic DNA) included mini-sequencing, restriction enzyme digestion, Sanger sequencing, Southern-blotting and/or multiplex ligation-dependent probe amplification(MLPA).

Results: We analysed 265 patients with CAH (65 with SW, 51 with SV and 149 with NC). In 211 patients (80%) the genotypes were in agreement with their phenotypes, while in the remaining 20%, only one pathogenic allele was identified or their genotype was normal. In the SW group the most frequent variant was the splicing mutation g.655A>G(28.5%), in the SV was g.999T>A(25.5%), and in NC was g.1683G>T(61%).

Conclusions: Knowing the molecular bases of CAH is essential for a correct genetic counselling; prenatal diagnosis and treatment during pregnancy can be offered to couples at risk of having a female child with SW or SV in order to avoid sexual ambiguity of the newborns.

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P13.14B

Microdeletion 1p36 diagnostic follow-up of a cohort of 70 patients diagnosed in France

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The purpose of our study was to review the prevalence of 1p36 deletion diagnosed in France in comparison with the same work made for 22q11 deletion (700 patients). The 1p36 deletion has a described incidence of 1/5000 to 1/10,000 births living. It is also a common condition within the microdeletions and potentially the most frequent after 22q11 deletion any age. Following a national ACLF survey with 15 centers, we report a cohort of 70 patients born living,at least 30 girls and 20 boys diagnosed between 2004 and 2017. The age of patients is 2 days to 31 years. The diagnosis was possible in the first years by the FISH technique, MLPA and finally thanks array-CGH in priority. This is a deletion interstitial in the majority of cases. For relatives tested more than 28 cases occurred de novo. Patients had clinical signs consistent with those already described in literature. They had growth retardation, hypotonia and/or a delay in acquisitions, or Intellectual disability (51/66 responses), IUGR and/or stunting (32/32 responses), a facial dysmorphism (53/56). Cardiac malformations or large vessels (> 33/53 responses), seizures (29/36 responses), brain abnormalities (30/43 responses), behavioral disorders (15/16 responses). Children were too young to have all signs. The size of the deletion evaluated in most cases and ranged from 4100bp to 47Mb. Two groups with a distal or proximal deletion were detected. This study is not exhaustive but raises the need to create registers for a better evaluation of the prevalence of microdeletions for better management.

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P13.15C

Partial trisomy 21 map: ten cases further supporting the highly restricted Down syndrome critical region (HR-DSCR) on human chromosome 21

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Background: Down syndrome (DS) is characterized by the presence of an extra full or partial human chromosome 21 (Hsa21). An invaluable model to define genotype-phenotype correlations in DS is the study of the extremely rare cases of partial (segmental) trisomy 21 (PT21). A systematic retrospective reanalysis of 125 PT21 cases allowed the identification of a 34-kb highly restricted DS critical region (HR-DSCR) as the minimal region whose duplication is shared by all PT21 DS subjects.

Material and Methods: We reanalyzed at higher resolution three cases previously published and we searched for any new PT21 report in order to verify whether HR-DSCR limits could prospectively be confirmed and possibly refined.

Results: Hsa21 partial duplications of three PT21 subjects were refined through array-CGH and resulted fully consistent with the previous reports and with the presence of a duplicated HR-DSCR only in DS subjects and not in non-DS individuals. Seven additional PT21 cases have been incorporated into the PT21 map. The PT21 map now integrates 132 subjects onto a common framework

fully consistent with the presence of a duplicated HR-DSCR, on distal 21q22.13 sub-band, only in DS subjects and not in non-DS individuals. No documented exception to the HR-DSCR model was found.

Conclusions: This prospective work further support the association of the HR-DSCR with the diagnosis of DS, representing an unbiased validation of the original model. Further studies are needed to identify genetic determinants presumably located in the HR-DSCR and functionally associated to the critical manifestations of DS.

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P13.16D

The activation of FGFR3 signalling has different consequences in the transmission of mutations the male germline

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The majority of new mutations originate in the male germline. However, to date we lack information on a unique type of mutagenesis—expansion of driver mutations in the male germline. These are associated with congenital disorders, occur at thousand-fold higher frequencies than other mutations, and drastically increase with paternal-age. To date, the mechanisms propagating these germline driver mutations are not completely understood. Here we examined the origin and expansion of four driver mutations in sperm and a dissected testis differing in mutation rates and the strength of dysregulation of the mutant FGFR3 receptor: c.1138G>A, c.1138G>C both causing achondroplasia (ACH), c.1948A>G associated with thanatophoric displasia II (TDII), and c.1948A>C causing hypochondroplasia (HCH). Only two out of four mutations (c.1138G>A and c.1948A>G) showed that mutant DNA concentrated within different clusters of the old donor's testis, resulting from the growth advantage of mutant stem cells caused by the activation of FGFR3. Interestingly, no measurable clustering was observed for the ACH mutation with the lower mutation frequency (c.1138G>C). We also observed that mutations with a stronger effect on FGFR3 signaling (TDII) showed a reduced transmission into sperm. In contrast, mildly activating mutations (HCH) were measured at a very high frequency in sperm of old donors by ultra-sensitive sequencing. Thus, there are important regulatory mechanisms at different developmental stages of spermatogenesis that affect the downstream transmission of driver mutations. Our analysis forms a basis for understanding this type of mutagenesis and the associated risks of delayed parenthood in our society. Funded by: LIT213201001 and FWFP25525000

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P13.18B

Functional analysis of sequence variants affecting splicing in Mendelian disorders

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Introduction: The development of Next-Generation Sequencing technology revealed that significant part of Mendelian disease-associated mutations is located in non-coding regions and may affect splicing. Because of the complexity of splicing regulation, it is not possible to predict accurately the effect of genomic variants on splicing events and RNA structure. In this work, we focused on functional analysis of genomic variants affecting splicing in a variety of Mendelian disorders.

Materials and Methods: To determine the effect of mutations we used two approaches: (1) RT-PCR from available patient's samples and (2) *in-vitro* minigene assay. For different cases, we performed one or both methods.

Results: We analyzed >25 previously uncharacterized genetic variants in >12 genes, associated with different Mendelian disorders. These variants are located in both exons and introns and mostly were classified as variant of unknown significance (VUS). We determined the effect of these variants on mRNA structure; it allowed us to classify most of them as pathogenic and to make assumption of the mechanisms involved in the molecular pathogenesis of diseases (e.g. RNA degradation by NMD, disruption of functional domain of protein). Additionally, we compared our experimental data with prediction tools for splicing events and revealed that it is not always possible to predict accurately the effect of mutation on splicing.

Conclusions: Although it is now known that mutations affecting splicing can cause the Mendelian diseases, however their contribution may be underrepresented due to limitation of diagnostic procedures. To prove the

pathogenicity of these mutations, additional functional analysis is often required.

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P13.19C

Interstitial microduplication Xp22.2 in two brothers with developmental delay and mild facial dysmorphism

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Interstitial microduplications affecting chromosome band Xp22.2 are very rare with only a few cases described so far. Reported symptoms include developmental delay, intellectual disability, and minor dysmorphism. While all affected individuals are males, carrier females usually have a normal phenotype, which suggests that intellectual disability is probably due to a dosage effect of one or more duplicated genes. However, due to the rarity of Xp22.2 microduplications, their heterogeneous size and variable localization the contribution of single genes to the clinical phenotype is difficult to evaluate.

Here we report on male siblings with mild to moderate global developmental delay, minor dysmorphism and measurements within normal ranges. Family history is unremarkable, both parents and the older sister are healthy. Molecular karyotyping revealed an interstitial microduplication Xp22.2-Xp22.31 of 5 megabases in both affected brothers (arr[hg18] Xp22.31p22.2(9301848_14415165)x2). The duplication, which was probably inherited from the healthy mother contains 36 OMIM-annotated genes, including *CLCN4*, *MID1*, *HCCS*, *ARGHAP6*, *FRMPD4*, and *OFD1*. Hemizygous loss of function mutations of *CLCN4* (encoding the chloride/hydrogen ion exchanger ClC-4) and *FRMPD4* (encoding a neural scaffolding protein) cause X-linked intellectual disability type MRX15 and MRX104, respectively. Both genes are highly expressed in brain and undergo X inactivation in females. It is tempting to speculate that *increased expression* of genes like *CLCN4* and *FRMPD4*, which are subject to dosage compensation, may contribute to intellectual disability in males with X chromosomal duplications. Analysis of further patients with overlapping Xp microduplications will be required to elucidate the contribution of these genes to the clinical phenotype.

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P13.20D

MicroRNAs as the link between Ribosomal Proteins Regulation and Colorectal Cancer: A promising therapeutic target

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Colorectal cancer (CRC) is the third leading cause of death in the world. Due to its slow development from pre-malignant lesions, perspectives to reduce the burden of disease by early detection and treatments are particularly promising for this disease. Although, a list with the cancer-related miRNAs has been created, their role in CRC remains to be elucidated. There appears to be a clear link between Ribosomal Proteins (RPs) and cancer as deregulation of RPs was shown to interfere with basic biological processes such as cell cycle regulation, apoptosis, genome integrity and tumorogenesis. This attribute requires a tight and careful regulation of RPs expression which might tend to cycle through carcinogenesis and cancer progression. We hypothesize that this cycling is at least partially controlled by miRNAs. Preliminary in silico analysis demonstrated that five miRNAs (miR-129-5p, miR-19b-1-5p, miR-193b-5p, miR-1207-5p, miR-663a) regulate more than 85% of the human RPs turning those miRNAs into potential crucial players in RP expression regulation. Interestingly, those miRNAs appear to also regulate genes that are involved in proliferation and cancer related pathways. Expression analysis in a panel of CRC cell lines with increasing aggressiveness, demonstrated differential expression of the above miRNAs. The aim of this project is to explore the connection between RPs and miRNAs in relevance to CRC, with the goal to expose promising miRNA therapeutic targets at different stages of carcinogenesis. Furthermore, it is expected that alternation of miRNA expression in colorectal tissue through an exosomal-based drug delivery system will improve disease outcome.

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P13.21A

Genetic heterogeneity & di/oligogenic inheritance involvement in variable expressivity of Noonan syndrome

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Noonan syndrome (NS) is characterized by an autosomal dominant inheritance, variable expressivity and genetic heterogeneity, given the high number of variants in PTPN11, SOS1 RAF1, NRAS, KRAS, BRAF, MEK1 and SHOC2 genes. Despite the increasing application of NGS in 30% of patients pathogenetic mutations remain unknown. Further NS genes and pathogenetic mechanisms are expected. We search for new NS genes by a targeted NGS analysis using a panel of 26 RAS pathway genes, in nine NS patients negative after genetic screenings. New potentially pathogenetic variants were detected in NS genes: c. T355C (p.Y119H) in LZTR1 and c.A2882G (p.D961G) in A2ML1 genes. We also observed in the same patient mutations in A2ML1 (p.K110T) and SOS2 (p.Q742X) and identified two new NS candidate genes in three patients. All of them have healthy parents and inherited a missense mutation in a new NS gene from one parent and the second one in a known NS gene from the other parent. The co-presence of both mutated genes probably contributes to the NS phenotype. Functional studies on lymphoblastoid cells from the three trios are ongoing. We propose an additive effect of subclinical mutations leading to the RAS pathway activation, causing NS. According to this hypothesis NS could be classified as mono/di/oligenic disease, a model that could explain, with genetic heterogeneity, the variable expressivity. Subclinical expression of specific variants, escaping unfavorable selective pressure, show high allelic frequency and therefore could be co-inherited. This model could also explain the missing of pathogenetic mutation in 30% of NS patients.

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P13.22B

Genomic knowledge as the powerful tool to understand the obesity

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Obesity, with its complications, emerges as a major contributor to the global health burden assuming the status of a pandemic. It's extremely complex disorder resulting of interaction of biological, social and behavioural factors that cause increase in food intake and reduction in energy expenditure. Although rare monogenic forms, several genes and regions of susceptibility have been described, the genetic causes underlying remain largely unknown, despite the role of genetic background is indisputable. GWAS revealed consistent association between SNPs with BMI and fat-mass, but cannot demonstrate the undoubted causality, and elucidating the culprit events continues to be challenging, especially when it's not known the way in which variants primarily act. To expand our knowledge, we performed WES in 30 strictly clinical classified Caucasian probands, with severe early-onset obesity. We screened a set of 80 genes responsible/susceptibility for syndromic/monogenic forms, including pathways of obesity development. We identified potentially pathogenic variants in 75%. 5 cases presented a single variant in genes related to increase of BMI/WHR, 3 patients had variants in hypothalamic leptin-melacortin pathway, whereas remaining cases showing complex genetic background with substitutions in 2/more genes. Interesting, in light of the genetic background we planned personalized treatment in patient with family history of diabetes, hepatic steatosis and severe obesity who presented pathogenic variant in *SH2B1*, leaded significant weight loss. The systematic discovery of rare variants in complex diseases suggests that the reverse-strategy is fruitful for assigning pathogenic effects of several genes simultaneously: the genotype-first approach will be able to identify clinically recognizable phenotypes

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P13.23C

Atypical recombinant chromosomes arising from parental paracentric inversions: report of three patients

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Introduction: Paracentric inversions can generate aneusomic gametes with duplication-deficiency distal to the inversion breakpoints and the formation of unstable dicentric and acentric derivatives leading to low embryonic viability. We report atypical recombinant monocentric chromosomes arising from parental paracentric inversions in three patients with syndromic intellectual disability.

Materials and Methods - Results: Chromosomal microarray showed similar rearrangements characterized by interstitial duplications-deficiencies proximal to the breakpoints of a parental paracentric inversion. The study of genotypes in the three patients using SNP microarray showed that: (i) the parental origins of the duplications and the deletions were from the parents with the paracentric inversions; (ii) there were three haplotypes in the duplications showing that the gain of copy came from both homologous chromosomes of the parent with the inversion; (iii) for each patient, there was a copy neutral region between the deletion and the duplication, flanked by low copy repeats causing reported cryptic inversions. We thus hypothesized the presence of an additional cryptic paracentric inversion in the same parent, either on the chromosome bearing the initial paracentric inversion, or on the homologous chromosome. In both cases, the speculated mechanism may involve a crossingover within the small loop of a double inversion loop.

Conclusions: It is currently thought that heterozygotes for paracentric inversions have a risk of abnormal offspring comparable to that of the general population, and that prenatal diagnosis should not be offered systematically. The observations of this study have called this dogma into question and may guide future recommendations for genetic counseling.

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P13.24D

A complicated WES diagnosis: hereditary spherocytosis due to autosomal recessively inherited mutation in *EPB42* associated with mosaic genome-wide paternal uniparental isodisomy

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Introduction: Genome-wide uniparental disomy is very rare phenomenon. In over 10 patients described so far the manifestations were mostly due to known imprinting disorders. We report a case of a child without imprinting defects features with mosaic paternal genome-wide uniparental isodisomy (GWUPiD) and symptomatic autosomal recessively inherited mutation in *EPB42*.

Materials and Methods: 3y old girl was referred for whole exome sequencing (WES) due to chronic anemia, chronic liver failure, cysts of the bile ducts treated by Kasai procedure. After delivery, she developed jaundice, anemia and hepatopathy. Many metabolic disorders were excluded. WES (DNA from blood, repeated 3 times, also from independently collected blood sample) was performed on HiSeq1500. To identify GWUPiD a panel of forensic STRs (short tandem repeats) was analyzed.

Results: Bioinformatics analysis of WES revealed lower than average number of variants. We also observed "pseudo" homozygous variants with low percentage of reference allele (~15%), in particular we found p.(Arg310Gln)/c.929G>A in *EPB42* (associated with spherocytosis) at 85% level mosaic (confirmed by amplicon deep sequencing). The variant was observed in father's sample in heterozygous state. STR analysis confirmed GWUPiD in patient's blood with biparental inheritance (BPI) presented

at low level. DNA extracted from buccal cells, nails, hair follicles and urine sediment showed normal BPI.

Conclusions: To the extent of our knowledge this is the first case of a recessive disease occurring in the mechanism of GWUPiD diagnosed by WES.

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P13.25A

Repetitive elements associated with breakpoints of distal 5p deletions suggest mechanisms mediating these rearrangements

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Introduction: Cytogenomic techniques, such as single nucleotide polymorphism (SNP) microarrays, allow the detection of copy number variants and structural characterization of breakpoint sites in several genomic diseases. Mechanisms have been proposed to explain genomic rearrangements, including nonallelic homologous recombination (NAHR), nonhomologous end joining (NHEJ), replicative mechanisms and long interspersed element (LINE)-mediated retrotransposition.

Material and Methods: We used Illumina Infinium CytoSNP-850K and UCSC Genome Browser (GRCh37/hg19) in order to map the breakpoints in 14 patients with 5p distal deletion.

Results: The results revealed breakpoint patterns on chromosome 5 ranging position chr5: 17,235,998-34,402,152. The structural characterization of breakpoints reveals that 10 of the 14 cases presented predominantly LINEs than SINEs. We only detected one patient with large low copy repeats (LCRs >10 kb), having 98 - 99% similarity.

Conclusions: The most common type of SINEs are *Alu* elements that are associated with NAHR and breakpoints with large LCRs (>10 kb) with high sequence homology also promote NAHR. Furthermore, we can assume that other mechanisms may be involved in the stabilization of DNA double-strand breaks in these cases, as NHEJ, that result in non-recurrent rearrangements leading to variation in deletions sizes and breakpoints location. The breakpoints investigated were in regions with repetitive elements, with the exception of two samples, suggesting an important role in mediating terminal rearrangements, previously seen in other terminal deletions but not reported in 5p distal deletions. The elucidation of the breakpoints can suggest mechanisms underlying structural variants involved in terminal deletions syndromes. Grants: FINEP-CT INFRA 0160/12 SP8, FAPESP 2016/09452-0

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P13.26B

Functional retrogene at the RB1 locus

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Retrotransposons are a major class of mobile elements accounting for 45% of our genome. The DNA of a retrotransposon is transcribed into RNA then reverse-transcribed into a cDNA copy which is reinserted into the genome at a new location. Retrotranspositions are frequent and usually lead to inactive elements due to the lack of promoter. However, we report a rare phenomenon of retrotransposition leading to a functional retrogene, segregating in a retinoblastoma family. A father and his son were affected by retinoblastoma, a malignant tumor of the eye due to *RB1* mutation. Given the absence of *RB1* mutation by DNA sequencing, we embarked upon RNA analyses demonstrating an abnormal *RB1* transcript including 2 exons of the *HPF1* gene. Oddly enough, *HPF1* is located on another chromosome. Following genomic analyses showed the insertion of *HPF1* full cDNA into the large intron 17 of *RB1*. This insertion was present in the two affected patients and not in unaffected individuals from the family. Hence the retrotransposition of the *HPF1* gene into *RB1* leads to a chimeric fusion transcript *RB1-HPF1-RB1* by using new splice sites. The phenomenon keeps the frame but disrupts a

major functional domain of pRb and is likely to cause retinoblastoma. As the grand parents are unaffected, it suggests a recent retransposition e.g. in their gametes. Beyond the identification of the hidden causal mutation, we describe here a very rare phenomenon as functional retrogenes have an estimated frequency of one retrogene per million year [Marques et al. PLoS Biology 2005].

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P13.27C

Clinical and molecular characterization of an almost complete ring chromosome 4 in two sisters, with recurrence due to gonadal mosaicism

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Introduction: Autosomal ring chromosomes are rare cytogenetic findings that arise from breakage and fusion of the chromosome ends. Rings are mitotically unstable, usually sporadic, and associated with a "ring chromosome syndrome", characterized by a variable phenotype of growth retardation, few or no minor anomalies, and intelligence ranging from normal to moderate intellectual disability. We describe the clinical features and molecular characterization of two sisters with ring chromosome 4.

Materials and Methods: Karyotype analysis was performed on both sisters and parents. Chromosome microarray was performed on both sisters to delineate the imbalance at the breakpoints. Clinical correlation including physical examination and formal neurodevelopmental assessments are compared for both sisters, as one sister received growth hormone therapy.

Results: Both sisters had a large ring 4 chromosome in the majority of cells analyzed on karyotype (97% and 83% respectively). Microarray results were identical in the sisters, showing a 55.8 kb duplication on the terminal 4p arm and a 1.5 Mb deletion on the terminal 4q arm. No genes of interest were identified in these regions. Parental karyotypes on lymphocytes and fibroblasts were normal, with no finding of mosaicism for the ring 4 chromosome. Polymorphic marker analysis revealed maternal origin of the ring.

Conclusions: We describe the clinical features and molecular imbalances of two teenage girls with almost complete ring 4. To our knowledge, this is the first reported instance of a ring 4 chromosome recurring in siblings after extensive testing on parents, which suggests this was due to maternal gonadal mosaicism.

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P13.28D

Exploring by whole exome sequencing patients with initial diagnosis of Rubinstein-Taybi syndrome: the interconnections of epigenetic machinery disorders

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Background: Rubinstein-Taybi syndrome (RSTS) is an autosomal dominant neurodevelopmental disease affecting 1:125,000 newborns characterized by intellectual disability, growth retardation, facial dysmorphisms and skeletal abnormalities. RSTS is caused by mutations in genes

encoding for writers of the epigenetic machinery: *CREBBP* (~60%) or its homologous *EP300* (~10%). However, no causative mutation is identified in up to 30% of patients.

Methods: To identify novel candidate genes for RSTS, we performed whole-exome sequencing (WES) on eight individuals with a diagnosis of RSTS who had normal high-resolution array CGH testing and were *CREBBP*- and *EP300*- mutation-negative.

Results: In four families, we identified putatively causal variants in three genes (*ASXL1*, *KMT2D* and *KMT2A*) encoding members of the epigenetic machinery known to be associated with the Bohring-Opitz, Kabuki and Wiedemann-Steiner syndromes. Each variant is novel, arose *de novo* and was predicted to result in loss-of-function. In the remaining patients additional candidate variants in *XRN2* or in *PLXNB2*, not yet related to any human disease, and in *XYLT2* or *PLCB4* associated respectively to spondyloocular and auriculocondylar type 2 syndromes are identified.

Conclusions: These results underscore the broad clinical spectrum of RSTS and other Mendelian disorders of the epigenetic apparatus. The overlapping features of distinct intellectual disability syndromes herein underlined reflect common pathogenic molecular mechanisms affecting the complex regulation of balance between open and closed chromatin.

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P13.29A

ER stress as pathogenic mechanism in Spinocerebellar Ataxia 38 (SCA38)

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ELOVL5 gene is associated with autosomal dominant Spinocerebellar Ataxia 38 (SCA38, MIM#611805), a rare adult-onset cerebellar neurodegeneration. This gene encodes for an elongase, an enzyme localized in the endoplasmic reticulum (ER) where it is involved in the synthesis of a subset of polyunsaturated fatty acids. We explored pathogenic mechanism of SCA38, studying aberrant ELOVL5-p.Gly230Val protein.

We demonstrated a subcellular mislocalization in perinuclear area of aberrant protein in different cellular models.

Based on these, we hypothesized p.Gly230Val-ELOVL5 is a misfolded protein able to activate the cellular unfolded protein response (UPR). Supporting that idea, we showed a significant increase of ELOVL5 protein in SCA38 fibroblasts after a treatment with the proteasome inhibitor MG-132. In COS7 cells stably expressing p.Gly230Val ELOVL5 we demonstrated the activation of ER-stress response by a significant increase of UPR markers CHOP, ATF-4 and XBP1 and an alteration of ER homeostasis by a slow-down protein transport from ER to the Golgi. Moreover, the use of the chemical chaperone PBA, acting on unfolded p.Gly230Val-ELOVL5 in COS7 cells, led to a physiological ER relocalization of the protein. To determine whether the activation of UPR was associated with neuronal degeneration, we measured cell viability of primary cortical neurons overexpressing wild type or aberrant ELOVL5. Preliminary data suggested a slightly increased of cells death in p.Gly230Val ELOVL5 neurons. In conclusion, our results support a role for altered ER-stress response in SCA38 pathogenesis, suggesting chemical chaperones might be useful in the treatment. Telethon Grant: GGP14225

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P13.30B

Adult-onset beta-thalassemia intermedia caused by a 5 Mb somatic clonal segmental deletion in hemopoietic stem cells involving the *HBB* locus

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Heterozygous beta-thalassemia individuals, inheriting a single defective allele, are usually asymptomatic, but in extremely rare cases a transfusion dependent beta-thalassemia intermedia develops later in life. We report a rare case of late-onset thalassemia intermedia caused by inheritance of 1 thalassemic *HBB* variant along with an acquired somatic deletion of the normal trans *HBB* locus in peripheral blood cells, resulting in hemizygosity for the beta-thalassemia mutation in erythrocytes. Direct Sanger sequencing characterized the beta-thalassemia mutation in HBB (*HBB*:c.315+1G>A) in DNA isolated from leucocytes, buccal cells and hair. Leucocyte DNA was analysed for genomic copy number variations (CNVs) using the Affymetrix CytoScan HD Array with Chromosome Analysis Suite (ChAS, version 3.0) and software, according to the manufacturer's instructions (Thermo Fisher Scientific, Santa Clara, CA, USA). Sanger sequencing of leucocyte DNA gave a skewed ratio of normal (G) versus variant (A), although DNA from buccal cells and hair showed classic heterozygosity. Array analysis showed a 4.97 Mb deletion (hg19/GRCh37: 1,313,791-6,287,277) on the short arm of chromosome 11 of maternal origin, which included the patient's normal beta-globin gene cluster, causing hemizygosity of the paternally inherited beta-thalassemia mutation, explaining the predominant hemizygosity for the beta-thalassemia mutation and her evolving clinical phenotype. Typical heterozygosity for the beta-thalassemia mutation in DNA in other tissues (buccal cells, hair) suggests a somatic origin of the segmental deletion affecting the hemopoietic stem cells. The late onset of expression of beta-thalassemia intermedia indicates a preferential survival of the hemopoietic cells containing the deleted region, which contributes to the majority of erythropoiesis.

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P13.31C

Chromosome arm scale de novo genome assemblies better detect and resolve structural variation and chromosomal abnormalities related to and causing genetic disease

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Current methods for detection of balanced structural variation can be broken down into two categories: traditional cytogenetics and molecular methods. Cytogenetics may include chromosomal karyotyping, fluorescence *in situ* hybridization (FISH), chromosomal microarray and adaptions of them. Molecular methods primarily include NGS sequencing based methods. Bionano genome mapping, an optical mapping approach, is a method that combines the advantages of different categories while solving many of the limitations. Compared to cytogenetics, Bionano mapping is high throughput and removes manual interpretation, it also has much higher resolution, detecting balanced events as small as about 30 kbp compared to multi-megabases needed for cytogenetic approaches, and unbalanced events starting at 500 bp. NGS based methods often are limited by read lengths that cannot provide unambiguous information across repeat elements longer than individual reads. This limitation results in significantly reduced sensitivity for balanced variation and abnormalities as well as for insertions and even some categories of larger deletions. This is particularly true in highly medically relevant locations where segmental duplications mediate chromosomal abnormalities.

Genome mapping using Bionano Genomics' Saphyr System and new direct labeling chemistry (DLS) facilitates economical whole genome and highly sensitive structural variation detection. We present genome assemblies of individuals with genetic disease caused by extremely long tandem repeat array expansion and collapse, and by rearrangements mediated by large segmental duplications that can be completely resolved by Bionano mapping. Bionano mapping is a fast and cost effective method for the detection of a broad range of traditionally refractory SVs across the human genome.

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P13.32D

Unbalanced *de novo* translocations are formed by inverted duplications on derivative chromosomes

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Introduction: It has now been estimated that in 0,23% of patients with developmental delay and intellectual disability unbalanced *de novo* translocations are detected. The mechanisms underlying unbalanced *de novo* translocations have been intensively studied, but still enigmatic.

Materials and Methods: We have analyzed 5 de novo translocations, ascertained either by conventional GTG-banded chromosome analysis or by array analysis, confirmed with targeted FISH. All samples were analyzed by multicolor banding (MCB) FISH technique with probe sets XCyte for chromosomes 4, 8, 9, 10, 12, 13, 18, 21 and X.

Results: The translocations were present in all cells. FISH with corresponding subtelomeric DNA-probes confirmed the deleted and duplicated segments previously found on arrays. MCB pattern indicated inverted duplication of the terminal region of the centric segment of derivative chromosome in all cases. Inverted duplications were not detected on translocated segments in any of the cases.

Conclusions: Our findings suggest that unbalanced *de novo* translocations are formed by inverted duplications on derivative chromosomes. A "fold-back" mechanism of large inverted duplications formation may be the first step of *de novo* translocation origin. Following end-joining between of free end of the inverted duplication and another chromosome would give rise to an inverted duplication translocation chromosome.

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P13.33A

Skewed X inactivation seems to regulate the *GTPBP6* expression and be associated with neuropsychological performance in Klinefelter patients

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Introduction: Klinefelter syndrome (KS) displays a broad reproductive and endocrinological spectrum, in which the most common karyotype is 47,XXY. KS patients may present dysmorphological features and develop neuropsychological deficits, including intellectual disability (ID).

Materials and Methods: In this study, we analyzed: (1) expression of eight X-chromosome genes; (2) X inactivation pattern by the HUMARA technique, and (3) the neuropsychological IQ using Wechsler Adult Intelligence and Raven General Matrix scales.

Results: Eleven out of 13 KS patients selected for this work presented karyotype 47,XXY, one 49,XXXXY and another 48,XXYY. Among the genes analyzed, *XIST* (Xq13.2) and *GTPBP6* (Xp22.33/Yp11.32) showed differential expression in peripheral blood by RT-qPCR. As expected, *XIST* differed between all patients with KS and male controls (P=0.0046). Differently, *GTPBP6* had a higher expression in all KS patients than controls (P=0.0005, females; P=0.0167, males). Among these, 46.1% (n=6) present ID, in which the *GTPBP6* expression is also high (P=0.0012, females; P=0.0152, males). In addition, seven out of 13 KS patients revealed informative HUMARA tests. All four KS patients, with IQ value above 79 (IQ above the borderline), showed skewed X inactivation (SXI) but *GTPBP6* expression did not differ between controls. In contrast, in three KS patients, whose IQ values were below 75 (low IQ), was observed random X inactivation (RXI) and the *GTPBP6* expression was increased (P=0.00167, females; P=0.00476, males).

Conclusions: These data suggest the trend that SXI can negatively regulate the *GTPBP6* expression and, therefore, likely improves the neuropsychological performance of KS patients.

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P14 New diagnostic approaches, technical aspects & quality control

P14.001A

Ultra-exome:a new approach to solve the unsolved

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Trio WES-negative patients may carry very elusive variants that require additional testing. Trio WGS, high resolution array CGH, as well as transcriptomic studies can detect a part of missing variants. However, some structural information is lost anyway and the cost/workload of these additional approaches may be limiting factors. The recently developed linked-read sequencing technology from 10XGenomics combines a novel barcoding strategy with Illumina sequencing. Genomic DNA fragments are partitioned into separate micro-reactions, where the same index sequence is incorporated into each of the sequencing fragment inserts derived from a given long fragment. However, WES provides little information for genes with long introns. To overcome this limitation, we redesigned our WES capture target including spaced polymorphic regions located in deep introns (>30kb). These WES barcoded reads contain linked exons with long-range sequence information that is advantageous for identification intragenic structural variants and phasing. In addition, we are able to distinguish very similar genes, such as SMN1 and SMN2. We are applying this technology to solve the WES-negative patients affected by a number of different genetic disorders

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P14.002B
Increased diagnostic yield with exon-focused array platforms

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Array-Comparative Genomic Hybridisation (Array-CGH) is the first-tier genetic test for patients with unexplained developmental delay, intellectual disability, autism spectrum disorders or multiple congenital anomalies. Although pathogenic genomic imbalances are detected in a substantial subset of this group (15%-20%), the underlying cause of the phenotype cannot be determined in almost 50% of patients (Miller et al., 2010).

In order to increase the diagnostic yield of array-CGH, platforms are being adjusted in accordance with the discovery of novel disease-causing genes. Moreover, an increased probe density across exons enables the detection of exonic copy number variants (CNVs) in addition to whole gene or multiple exon variation.

We present six cases for which a pathogenic variant could be detected by means of the CytoSure™ Constitutional v3 microarray platform (Oxford Gene Technology), while this CNV was under the detection limit of the previously used v2 platform. The v3 platform combines the most up-to-date content information from ClinGen and the DDD project with exon-focused probe design for single-exon CNV resolution in disease-relevant genes.

We show that the use of an exon-focused array design results in an increased diagnostic yield, which will even further increase with the implementation of whole genome sequencing, as this enables the simultaneous detection of CNVs and single nucleotide variants (SNVs) for this group of patients.

Miller DT, Adam MP, Aradhya S, Biesecker LG, et al., Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet. 2010 May 14;86(5):749-64.

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P14.003C
Training a Facial Analysis Software to Recognize a Very Rare Condition: Aymé-Gripp Syndrome

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Introduction: Subjective impressions of an identifiable facial phenotype arise during clinical practice. It is challenging to assess this impression objectively, particularly for very rare syndromes. Aymé-Gripp syndrome (AGS) is such a rare condition, resulting from specific *MAF* mutations and characterized by developmental delay and ID, cataracts, hearing loss, short stature, pericardial effusion and skeletal anomalies. We evaluated an automated facial analysis tool for its ability to distinguish between patients with AGS and hundreds other rare genetic syndromes.

Methods: Frontal facial photographs of 13 individuals with molecularly confirmed AGS were used to train the Face2Gene (FDNA Inc, USA) tool against 216 other syndromes. In addition, we compared to images from 20 unaffected individuals and 20 individuals with Down syndrome (DS), chosen for its similarities to the facial features of AGS. Classification statistics were used, Receiver Operator Characteristic (ROC) curves and their Area Under the Curve (AUC) calculated.

Results: The training of the technology yielded and AUC of 0.97 which is above the significance level threshold

required by the tool and AGS is now included as a recognized condition. Comparison between the 3 cohorts yielded an accuracy of 89.1%, double the random accuracy of 37.7%. In binary comparisons, the analysis correctly differentiated between AGS and controls with an AUC of 0.99 (STD 0.01).

Conclusions: Even few images can be sufficient for an automated facial analysis tool to identify characteristic facial features of rare syndromes such as AGS, and thus allow for targeted molecular testing if clinically indicated.

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P14.004D

NUCLEIC-CARD™ as innovative blood collection device for NGS genetic analysis

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Background: Clinical genetic analysis and research, including Next-Generation Sequencing (NGS), have increased in recent years. Fresh blood has been always considered the gold standard, but it is prone to handling and biological risks during shipping. Copan developed the NUCLEIC-CARD™ (NC) for collection, preservation, stabilization, transport and long-term storage at room temperature of blood samples. The objective of this study was to report the performance of the NC, as analytical accuracy of clinical (~6,300 clinically relevant genes) and whole (WES) exome sequencing of DNA extracted from blood spots on NC.

Methods: DNA, extracted from the entire NC blood spot of 16 donors using a modified QIAamp® DNA mini kit with the Copan NAO® (nucleic acid optimizer) baskets protocol, were used for this study. Besides clinical exome analyses, two of the samples were also used for WES.

Results: High-throughput sequencing, performed on DNA extracted from dried blood spots onto NC gave excellent yield, reaching high quality levels, comparable with the ones obtainable from EDTA-blood samples. Also, it has been possible to achieve overlapping values in terms of final average coverage on target, target percentages at 10x, 20x and 30x, and base call quality.

Conclusions: Data obtained in this study demonstrated that good quality DNA can be recovered from blood spotted on Copan NUCLEIC-CARD™, and that such DNA is perfectly suitable for high-throughput analyses such as clinical and whole exome sequencing. NUCLEIC-CARD™ allows long-term storage and stability of dry blood samples for easy samples shipment at room temperature to testing laboratories.

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P14.005A

Genetic testing approach for patients with congenital anomalies of the kidney and urinary tract (CAKUT)

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Introduction: Congenital anomalies of the kidney and urinary tract (CAKUT) are a heterogeneous group of embryonic malformations representing the most common cause of chronic kidney disease in the first 3 decades of life. About 40 monogenic causes of CAKUT are known explaining <20% of CAKUT cases. We aim to improve the genetic diagnosis of this nephropathy.

Methods: Ninety unrelated patients with CAKUT (21% with perinatal death) mostly bilateral (86%), with a suspicion of monogenic cause due to extrarenal defects (29%) and/or positive familial history of CAKUT (46%) underwent a two-step genetic analysis. 1) *HNF1B* gene mutation screening, for CNV and SNV; and if negative; 2) targeted next generation sequencing (NGS) of a >200

kidney-disease gene panel including 62 genes associated with CAKUT.

Results: Causative mutations were identified in 38% (34/90) of patients [9% (3/34) with perinatal death] involving the following genes: *HNF1B* (27), *PAX2* (5) and *ACE* (3). Probably pathogenic variants were detected in 3% (3/90) of patients in *JAG1, NEK8* and *DYNC2H1* genes. Variants of uncertain significance were detected in 8% (7/90) of patients in the: *SIX2, RET, PAX2, GLI3, NOTCH2* and *PAX8* genes.

Conclusions: *HNF1B* screening allowed the etiologic diagnosis in one-quarter (27/90) of our CAKUT patients, of whom only one presented with perinatal death. NGS of our kidney-disease gene panel identified a likely pathogenic variant in an additional 12% (11/90) of patients. Our approach achieved a 42% diagnostic yield (63% with positive familial history/extrarenal defects). Whole exome sequencing is undergoing in selected cases.

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P14.006B
Determination of optimal call quality cutoffs for clinical applications of NGS

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Introduction: NGS generates large number of variants of varying quality. It is important to decide which calls to retain for downstream, but few formalized, practical recommendations exist on how to do it.

Materials and Methods: We sequenced two platinum genome samples, NA12877 and NA12878, using Agilent FocusedExome enrichment kit on Illumina HiSeq 2500 platform. The data was called using in-house pipeline based on GATK Best Practices. We tested multiple combinations of quality metrics (depth of coverage, B allele count, B allele fraction, and HaplotypeCaller's QUAL score) to filter the resulting calls. By comparing the remaining calls with platinum genome reference callsets, sensitivity and false positive rate were calculated for each combination of cutoff values.

Results: HaplotypeCaller's weighted QUAL field provides the most powerful metric for filtering. Sequencing depth (DP), while widely used, has relatively little to do with call accuracy. For Illumina HiSeq platform and GATK Best Practices-like processing pipeline, we report the optimal filtering parameters to be: DP≥6 & QUAL≥75. This set of cutoffs achieves a tradeoff by calling reasonably many variants (45 361 using targeted sequencing kit) while minimizing false positive rate (0.34%). In comparison, naive depth-only filtering (DP>12) called only 36 171 variants with a FPR of 0.71%.

Conclusions: We present a formal yet simple approach to determine the most optimal quality filtering cutoffs for NGS calls. This allows to get the most out of a NGS sample without compromising accuracy of the results.

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P14.007C
Germline analysis using FFPE-tissue in cancer families with high mortality rates

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The identification of disease-causing germline mutations in families with hereditary cancer is essential for predictive testing of unaffected family members and estimation of individual lifetime risks. Germline testing usually requires blood- or saliva-derived DNA of the index patient. However, due to high rate of mortality living affected family members may not be available in cancer families. The establishment of Next Generation Sequencing (NGS) in genetic diagnostics enables comprehensive risk gene analyses even in DNA isolated from formalin-fixed-paraffin-embedded (FFPE) tissue. Here, we show successful post mortem germline analysis in high risk breast (BC) and ovarian cancer (OC) families by using DNA from non-cancerous FFPE-tissue derived from tumor surgery. Of particular importance is the exact discrimination between tumor and surrounding healthy tissue because only analysis in non-cancerous tissue assures the identification of germline mutations. So far, in 111 families without a living index patient, we performed NGS using FFPE-derived DNA samples by employing the TruRisk® gene panel and identified a mutation prevalence of about 29 % within 15 analyzed cancer predisposition genes. This result is comparable to the mutation prevalence identified using blood-derived DNA in familial BC and OC cases. Based on our

experience, we highly recommend using non-cancerous FFPE material for germline analysis in families without a living index in a routine diagnostic setting. Particularly, in hereditary cancer families this procedure is of substantial relevance for subsequent predictive gene analysis, risk calculation and cancer prevention in further family members.

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P14.008D

Cell-free DNA analysis with the 2100 Bioanalyzer system

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Sequencing of cell-free DNA (cfDNA) extracted from blood specimens or other body fluids is possible due to the establishment of low input library protocols for next-generation sequencing workflows. Accurate quantification of cfDNA samples is essential to determine suitable input amounts for cfDNA library preparation prior to sequencing. The main component of cfDNA samples is the mono-nucleosome with a size around 170 bp, sometimes with additional species representing nucleosome multimers. Further, cfDNA samples may contain larger DNA fragments dependent on the donor's health status, preanalytical sample treatment, or extraction method. High molecular weight material can negatively influence library preparation and subsequently result in lower sequencing depth. Therefore, reliable quantification of cfDNA requires a method that separates DNA fragments by size, such as electrophoresis. This poster shows the use of a new cell-free DNA assay for the 2100 Bioanalyzer system as an add-on for the High Sensitivity DNA assay. The cell-free DNA assay enables automated cfDNA quantification with pre-defined regions. Moreover, the results include sample purity as a score to qualify cfDNA samples according to their contamination level with high molecular weight material. The features of the cfDNA assay are described with examples of typical sample patterns.

H. Mundi: A. Employment (full or part-time); Significant; Agilent Technologies LDA. **E. Graf:** A. Employment (full or part-time); Significant; Agilent Technologies. **R. Nitsche:** A. Employment (full or part-time); Significant; Agilent Technologies.

P14.009A

Detection of low-level mosaicism by array CGH using low amounts of DNA labeled with CYTAG SuperCGH labeling kit

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Background: aCGH is a powerful clinical diagnostic tool allowing the genome-wide assessment of CNVs with high resolution. Despite several studies validating aCGH for the detection of cytogenetic abnormalities, only a few have reported on array sensitivity. Here, we determined the sensitivity of whole-genome aCGH by labeling low DNA inputs with CYTAG® SuperCGH labeling kit and detecting low levels of artificial mosaicism.

Methods: Female genomic DNA from a patient with Jacobsen syndrome and from a single normal source were used in this study. Sensitivity was assessed by preparing different percentages of artificial mosaicism (5%, 8%, 10%, and 15%), labeling 50 ng of DNA with CYTAG® SuperCGH labeling kit (Enzo, ENZ-GEN120), and hybridizing the labeled DNA onto SurePrint® G3 human CGH 4x180k microarrays.

Results: DLRS were 0.115, 0.104, 0.102, and 0.105 for samples with 5%, 8%, 10%, and 15% artificial mosaicism, respectively. Female genomic DNA with Jacobsen syndrome has a 12.5Mb terminal deletion on the q arm of chromosome 11. With a setting for the Average Log Ratio in the CytoGenomics software of 0.0 or 0.05, the 12.5Mb deletion was visualized and detected in samples with as low as 8% of artificial mosaicism.

Conclusions: Upon labeling of 50 ng of DNA with CYTAG® SuperCGH labeling kit, the lowest limit of detection was deemed to be 8%. The results demonstrated that aCGH is an invaluable tool to detect segmental aneuploidy with length around 10Mb in mosaic samples with low DNA input thereby offering new possibilities for aCGH analysis in prenatal or oncology.

P. Pande: None. **J. Coleman:** None. **N. Rana:** None. **M. Mathieu:** None.

P14.010B

Integrated genetic analysis: Harnessing the power of NGS

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Introduction: The study of copy number variants is often equated to microarray platforms. Microarray platforms are

appropriate to detect genetic imbalances but present challenges that might be overcome by NGS technologies. We propose an NGS based approach to chromosomal analysis with an increased sensitivity and an unbiased representation of the whole genome.

Materials and Methods: We compare the results of microarray analysis and pair-ended, low-coverage whole genome sequencing to test 31 samples from cell lines and 19 deidentified individuals. All variants were verified by orthologous methods.

Results: The NGS-based approach identified all variants identified by microarray plus 52 additional copy number variants (CNVs). The NGS-based test exhibits increased sensitivity with a detection limit of up to 200bp when average sequencing coverage is 1X. Identification of exact breakpoints requires higher coverage. Furthermore, the NGS-based chromosomal analysis allows the identification of structural abnormalities that microarray is unable to detect. Primer design for segmental duplication is extremely difficult for areas of high homology, a limitation shared by microarray, MLPA or qPCR. In contrast, NGS-based chromosomal analysis and stringent bioinformatic protocols allow precise mapping of reads with up to 1 base difference in 300 bp DNA block (~99.7% identity) correctly computing CNVs in areas of segmental duplication.

Conclusions: Compared to microarray platforms, NGS-based chromosomal analysis exhibits an increased sensitivity and an option to streamline genetic testing. NGS-based CNV analysis can be part of a step-wise testing algorithm that includes multigene panels and exomes, resulting in a cost and time effective alternative to current strategies.

A.J. Obregon-Tito: A. Employment (full or part-time); Significant; Fulgent Genetics. **C. Lee:** A. Employment (full or part-time); Significant; Fulgent Genetics. **J. Li:** A. Employment (full or part-time); Significant; Fulgent Genetics. **M. Teguh:** A. Employment (full or part-time); Significant; Fulgent Genetics. **Y. Shen:** A. Employment (full or part-time); Significant; Fulgent Genetics.

P14.012D
High Yield of Clinical Exome Sequencing as a primary molecular diagnostic tool in Adults

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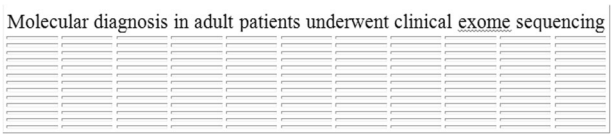
Introduction: Clinical exome sequencing (CES) is increasingly used as a molecular diagnostic tool for genetic disorders. We presented here the use of CES as a primary diagnostic tool in adults.

Methods: We analyzed adult patients consulted to the adult genetic service in 2017, who underwent CES.

Results: CES revealed a diagnosis in 10 of the 28 patients (35.7%) (age range: 20 to 104 years old) (Table 1). 14 pathogenic variants (PVs) or likely PVs were identified in 12 genes: *CPT1A,CPT2,MMAHC,CXCR4,ACVRL1, FANCA,PTEN,NF1,BRCA1,SLC26A2,HBB,TGFBR1*. Two patients carried two diagnoses (Patient 8 and 9 in table 1). Two patients (2/28 = 7.1%) with autosomal recessive disorders (Citrin Deficiency, Autosomal recessive Polycystic Kidney Disease with Hepatic Fibrosis) were found to harbor only one mutant allele in the causative genes (*SLC25A3* and *PKHD1* respectively). We also identified secondary findings in two cases (2/28 = 7.1%) without primary molecular diagnosis (Hb Constant Spring carrier and G-6-PD deficiency carrier). Furthermore, we identified the variants in the genes that strongly support the clinical diagnosis, but the pathogenicity cannot be confirmed solely by CES in 21.4% (6/28) of the patients.

Discussion: We report the high yield of diagnosis and secondary findings (14/28 = 50%) from CES in adult patients. The yield was increased to 71.4% (20/28) if all possible/actionable results were included. Our results demonstrated the efficacy of CES as a primary diagnostic tool in adults.



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P14.013A
Diagnostic yield and clinical utility of clinical exome sequencing

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Introduction: Clinical exome sequencing (CES) involves the sequencing and analysis of the coding regions of only the clinically-relevant genes in the genome compared to sequencing all 20,000 genes as in a whole exome sequencing (WES).

Aim: To assess the clinical utility of CES (CentoDx Plus™) in patients tested to date using diagnostic yield as a measure.

Methods: Previously reported 91 CES cases were analyzed to identify cases with diagnostic variants (pathogenic/ likely pathogenic) and those with variants of unknown significance (VUS).

Results: In 45 (49.4%) of 91 cases tested, CES resulted in a (likely) confirmed diagnosis. Additionally, in 25 cases (27.5%), one or more VUS were detected. Out of these, parental testing was recommended for 13 cases to help reclassify the VUS as it matched the phenotype of the patient. Seven (7.7%) patients were (likely) carriers of one diagnostic variant or VUS. Fourteen (15.4%) cases were negative and no phenotype-related variant was detected.

Conclusions: The high diagnostic yield of CES (49.4%) when compared to WES can likely be attributed to CES being used to diagnose cases with known, suspected diagnoses as well as undiagnosed cases with unknown, complex phenotypes. CES is a versatile test with high clinical utility for a variety of diagnostic indications. Due to its cost- and time-efficient nature and high diagnostic rate, CES is a good alternative to sequential testing, NGS panels or WES. Especially in regions with economic barriers to genetic testing, CES should be considered a prime test of choice.

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P14.014B

Clinical exome sequencing in 508 Middle Eastern families with Mendelian diseases provides a high diagnostic yield and discovery of novel genes

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Background: Clinical Exome Sequencing (CES) is rapidly becoming a standard molecular diagnostic tool in rare and diverse genetic disorders such as neurocognitive/neurodevelopmental, neuromuscular disorders, and multiple congenital anomalies. The clinical indications recommended for CES, the overall molecular diagnostic yield for diverse phenotypes, along with differences in molecular diagnostic yields between CES as first-tier test or reflex test, will be determined in a cohort of 508 patients from a highly consanguineous population.

Methods: A clinical cohort of 508 probands from Qatar underwent singleton or trio CES from April 2014 to December 2016. Cases were considered 'solved' when mono- or bi-allelic pathogenic or likely pathogenic variants (collectively "PV"), including pathogenic CNVs, were present in a disease gene, consistent with the known mode of inheritance of a disorder.

Results: The CES diagnostic yield for our cohort of patients was 48.0% (n=245). Consanguinity and a positive family history predicted a higher diagnostic yield up to 56%. Eleven patients (2.1%) had two distinct genetic disorders. 18 novel candidate genes were identified. As 65% of families were consanguineous, a considerable number of probands (56%) were homozygous for a PV in a gene associated with an autosomal recessive trait, and another 9% had PV in genes with AR or autosomal dominant inheritance. Nevertheless, a considerable number of patients had PVs in AD (29%) or X-linked genes (5%), or *de novo* copy number variants (1.8%).

Conclusions: Due to its high diagnostic yield in this population group, CES is recommended as a first-line diagnostic approach in the genetics clinic.

T.I.M.M. Ben-Omran: None.

P14.016D

Copy Number Variations calling from Whole Genome Sequencing data as a substitute to array CGH

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Along with Single Nucleotide Variants (SNV), Copy Number Variations (CNV) are a major player in genetic diseases, and can be detected in up to 15% of patients with heterogeneous diseases. They can affect the coding part of the genome and exert a gene dosage effect, or be located in non-coding regions, where they can alter the DNA tridimensional organization and the enhancer-promoter contacts. If Whole Exome and Genome Sequencing are now classical high-throughput approaches for SNP detection, array-CGH remains the gold-standard for genome-wide study of CNV. Efficiently combining the detection of both types of variants with a single technique would prove to be a speed- and cost-effective approach towards the determination of the causative variant. In 27 index cases with limb malformations, we compare array-CGH results with calls from WGS with different algorithms, either based on split-reads and abnormal paired-end insert size (Delly), or on coverage variations (CNVnator, cnvkit, FREEC). With default parameters, 13 to 89% (on average 59%) of array-CGH deletions can be detected by at least one algorithm with 50% reciprocal identity. This is only the case for 0 to 45% of amplifications (on average 31%). Many false positive variants are also called, and filters, including cohort genotyping, need to be adapted to improve detection efficiency. Further tuning is hence required to reach an efficient pipeline in terms of both sensitivity and specificity, but CNV detection from WGS data is a promising alternative to array-CGH.

M. Coutelier: None. **M. Holtgrewe:** None. **M. Jäger:** None. **R. Flöttman:** None. **M. Mensah:** None. **M. Spielmann:** None. **P. Krawitz:** None. **D. Horn:** None. **D. Beule:** None. **S. Mundlos:** None.

P14.017A

Investigating the CNVs in routine diagnostics using WES and array in Brazilian patients

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Over the last years the identification of CNVs from whole exome sequencing (WES) data has become a common practice for research. In this study, we evaluated 32 patients with multiple congenital malformations and developmental delay with WES (Illumina) technique using the ExomeDepth software and with array (Illumina) using the BlueFuse software for CNVs evaluation. According to information from Illumina, the average of the minimum resolution for CNVs detection by array is about 18 kb, thus we selected only CNVs larger than this value to measure the consistency between the both methodologies. Array analysis for all patients showed 44 CNVs, among then 29 alterations were deletions and 15 were duplications. Exome analysis, of the same samples, showed 60 CNVs wherein 39 were deletions and 21 duplications. So, the overlapping rate of genomic changes was 13.8% between the techniques. The extraction of CNVs information obtained from the WES is an advantageous approach since it can improve the cost-effectiveness and reduce the number of genomic tests required to diagnosis. However, WES is not developed for the evaluation of CNVs, and shows a high rate of false-positive and false-negative results. On the other hand, the array is a gold standard technique with high accuracy for detection of deletions and duplications. Thus, to date, the screening by arrays allows yet a better detection of the CNVs making possible the conclusion of diagnosis for most patients, suggesting array performance present an outstanding level when compared to exome in routine diagnostics. Grants: FINEP-CT INFRA 0160/12 SP8, CAPES.

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P14.018B

Preliminary evaluation of a *CYP21A2* genotyping by NGS

amplicon method based on LR-PCR enrichment for *CYP21A2* and chimaeras

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Introduction: Congenital Adrenal Hyperplasia (CAH) related to *CYP21A2* gene is an autosomal recessive disorders affecting steroidogenesis.

Material and Methods: We present a cohort of 33 patients: asymptomatic (AP):9, clinical/biochemical phenotype (CBP):20 (15 with clear diagnosis), unknown phenotype (UPP):4.

All DNA samples were sequenced using NEXTflex® Congenital Adrenal Hyperplasia Amplicon Panel. In some samples: SALSA-MLPA P050-C1-CAH probemix and Sanger sequencing were performed.

Results: 11 samples showed a profile compatible with presence of multiple copies and chimeric alleles.

AP had a concordant genotype in 77,78% (7/9).

NEXTflex and Sanger sequencing had concordant results in 81,82% (9/11).

NEXTflex and MLPA data were concordant for IVS2-13 status in 68,18% (15/22) and for copy number status in 50% (11/22).

Genotype-phenotype concordance in CBP was obtained in 80% (12/15).

Conclusions: NEXTflex is a great method to detect chimaeras and multimodular RCCX state, whose detection was not possible by Sanger or MLPA. NEXTflex also potentially allows to distinguish multiple copies.

The discrepancies in AP and CBP could be due to allele dropouts, caused by the presence of a copy of *CYP21A2* on 3' end followed by *TNXA*, or by formation of super-secondary structures close to I2-13. These potential limitations will be checked by no LR-PCR.

It is necessary to perform further analyses (trio analysis, check with no LR-PCR, test other genes related to CAH) in complex or not explained cases.

This study points out the importance of a multiapproach analysis based on complementary genetic tests, clinical and biochemical data to give an accurate diagnosis of CAH.

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P14.019C

Copy number variant caller for gonozomal chromosomes

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Introduction: The clinical noninvasive prenatal testing (NIPT) is mainly focused on detecting whole-chromosome aneuploidies using low-coverage whole genome sequencing. Nowadays, advanced versions of NIPT are able to identify even various subchromosomal copy number variants (CNV) such as microdeletions and microduplications. However, these CNVs are being detected only on autosomal chromosomes, since it is quite challenging to normalize and train parameters on gonosomal chromosomes due to variable amount of reads mapped to gonosomal chromosomes according to fetal fraction and sex of the fetus.

Materials and Methods: We extended our in-house CNV detector with gonosomal CNV feature. The normalization on autosomal chromosomes is done per chromosome, while autosomes are normalized relative to all autosomes. This allows us to train and normalize the algorithm parameters and predict local CNVs on gonosomal chromosomes. Training was performed on 790 samples with more than 7M reads.

Results: In addition to artificial testing samples with introduced sex chromosomal microaberration, we were able to identify three subchromosomal aberrations on chromosome X in clinical samples. The lower limit of detection was 1.66Mb.

Conclusions: The CNV detection software currently used in NIPT can be extended to detect local aberrations on gonosomal chromosomes. However, due to the normalization only for single chromosome, it is unable to detect whole chromosome aneuploidies (monosomies, trisomies, ⋯) - a different method is employed to detect these. Moreover, large scale aberrations are indistinguishable from their counterparts - for example deletion of the whole short arm (p) is indistinguishable from a duplication of whole longer arm (q).

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P14.020D

Clinical utility of copy number variant (CNV) detection by whole genome sequencing (WGS)

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Background: We have recently completed clinical validation of ClinSV, an analysis pipeline that identifies regions of CNV utilising evidence from split-reads, discordant pairs and depth-of-coverage to obtain a comprehensive high confidence CNV call-set. Clinical validation was performed against clinical microarray, MLPA and NA12878 gold standard. No lower limit for size was applied, allowing for unprecedented detection of CNV. Analytical sensitivity as assessed against NA12878 was 86.6% and 99.1% for deletions <500bp and >500bp respectively. Results were 100% concordant for reportable variants previously detected by microarray and MLPA.

Aim: Increasing CNV detection resolution should increase diagnostic yield, but to our knowledge there are no published studies on the clinical utility of genome wide analysis of CNV below the resolution of microarray. To identify variants previously undetectable by other methods, and determine the clinical utility of high resolution genome-wide CNV analysis, we have begun a retrospective review of previously reported uninformative cases (n=161) using ClinSV.

Results: Initial assessment of a small number of previous negative WGS cases has yielded diagnostic CNVs including: 1kb homozygous deletion of SPG7 exon 6 in a patient with Spastic Paraplegia and a compound heterozygous stop-gain plus exon 1 deletion of DST in two deceased neonates.

Conclusions: Genome-wide CNV analysis has wider breadth and higher resolution than any other test available and increases the diagnostic yield of WGS. It is particularly relevant to those patients with previous uninformative genome and exome testing.

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P14.021A

Validation of circulating tumor DNA analysis in a diagnostic laboratory: pre-analytical factors and quality control

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We present the results of our technical validation process in establishing analysis of circulating tumor DNA (ctDNA) as a diagnostic tool in our laboratory.

Like most cells in our body, tumor cells shed DNA in the blood flow. Detection of ctDNA and analysis of its mutational content can provide invaluable information on the genetic makeup of a tumor, and assist oncologists in deciding on therapy or in following the evolution of residual disease. However, the low absolute amounts of circulating DNA, and the minor fraction of ctDNA within it, constitute formidable analytical challenges. A key step into ctDNA detection is to avoid any contamination with genomic DNA resulting from cell lysis. Several brands of specialized blood collection tubes are available to prevent leukocyte lysis, but we found that they are not equally efficient, depending on storage temperature and on time before plasma preparation.

We report our analysis of preanalytical factors pertaining to ctDNA analysis (collection tubes, transportation time and temperature) and our conclusions in terms of instructions being provided to prescribing physicians and medical

professionals. We also stress the importance of proper DNA quality control and compare several methods to this aim, including a differential amplicon length PCR technique which allows us to determine multiple QC parameters from minimal amounts of circulating DNA.

Altogether, these data should provide a useful practical guide to other diagnostic laboratories wishing to implement the assay of ct DNA in clinical practice.

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P14.022B

Gene testing in CYLD cutaneous syndrome: 5-year data from the first European service

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Patients with CYLD cutaneous syndrome develop multiple cylindromas, spiradenomas or trichoepitheliomas. The occurrence of these rare skin tumours that are related to the hair follicle has been associated with germline mutations in *CYLD*. We established a UK wide gene testing service to detect *CYLD* mutations in patients fulfilling testing criteria. Here we report outcomes of *CYLD* gene testing using Sanger sequencing of coding exons. We received a total of 56 referrals of novel probands for *CYLD* gene testing over 5 years. We detected pathogenic *CYLD* mutations in 39 (69%) cases. Of these, 17 were novel mutations. Predictive cascade testing was done in 13 further cases, of which 6 confirmed the familial mutation. Previous estimates of mutation positivity ranged from 44-85% depending on the phenotype of CYLD cutaneous syndrome. Here, using conservative inclusive criteria, we report a rate of germline exonic and splice site mutation of 69%. Gene testing served to confirm the clinical diagnosis, inform genetic testing of unaffected relatives and in one case to facilitate pre-implantation genetic diagnosis. At risk patients who were confirmed to be mutation negative were able to plan their families without the concern of disease transmission. In summary, we highlight the experience of a single national centre performing *CYLD* testing. The ability to solve the majority of cases by sequencing coding exons suggest that additional assays, required to detect copy number changes and rearrangements, can be reserved for the investigation of mutation-negative cases, thus allowing a more judicious use of resources.

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P14.023C

CFTR RealTime Panel using pre-plated LightSNiP assays, a good strategy for *CFTR* genotyping

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Pathogenic variants in the Cystic fibrosis (CF) transmembrane conductance regulator *(CFTR)* gene have been linked to CF, one of the most common life-threatening genetic diseases. We provide *CFTR* genotyping by "cascade" strategy. Initially, we test the most common CF-causing variants by *in-house* methods. In next step we use commercial kit (Elucigene® CF-EU2v1). These routine methods are followed by sequencing to detect rare and unknown *CFTR* variants.

Aim: To develop and validate a fast, accurate, cost-effective method for detecting prevalent *CFTR* pathogenic variants based on LightCyclerTechnology (Roche®) with SimpleProbe® probes (LightSNiP assays, TIB Molbiol).

Methods: We developed the CFTR RealTime Panel for use with LightSNiP assays enabling scoring of 11 prevalent *CFTR* pathogenic variants (newborn screening data). LightSNiP assays were designed by TIB Molbiol (Berlin, Germany). Panel contains LightSNiP assays pre-plated and dried-down for convenient storage and stability in LightCycler® 480 Multiwell Plates, 96. LightSNiP assays carried out RealTime PCR in the LightCycler®480 Instrument.

Results: From amplification and detection with specific probes by melting curve analysis, it is possible to obtain a visual discrimination of normal and pathogenic alleles in the homozygous and heterozygous status. The cost of the CFTR RealTime Panel and Elucigene® is 19€ and 46€ per patient, respectively. The detection rate of CF-causing variants is 86% and 90%, respectively.

Conclusions: Based on the features and the ease of use of SimpleProbe® technology, we suggest that the LightSNiP assay is a good strategy for detecting prevalent *CFTR* pathogenic variants to provide the clinician correct results in a short time.

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P14.024D

NGS-based approach to detect aneuploidies and large

chromosome aberrations in patients with congenital cytogenetic abnormalities

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Introduction: Approximately 25% of congenital disorders are caused by chromosome abnormalities. The conventional karyotyping methods such as G-banding and fluorescence in situ hybridization (FISH) have been used for the diagnosis of chromosomal abnormalities. However, they have several limitations including possible false-negative results, time-consuming, and requiring an expert's reading. We evaluated the low-coverage whole-genome sequencing (WGS) using a semi-conductor next generation sequencing technique for genome-wide detection of aneuploidies.

Materials and Methods: Genomic DNA was extracted from peripheral blood or buccal cells from 9 patients diagnosed with congenital chromosomal abnormalities by conventional cytogenetic method. WGS library preparation and low-coverage genome sequencing (0.01-0.04X) were performed using Ion Torrent platform. Aneuploidies were detected using Ion Reporter Low-pass whole-genome aneuploidy analysis pipelines.

Results: We detected whole-chromosomal and sub-chromosomal duplications/deletions from genomic DNA by low-pass sequencing. We also detected the deletion in the end of chromosome and duplication at the adjacent region in one patient. By comparison of the results from conventional (G-banding) and novel (low-pass WGA) method, the origin of unknown fragment attached to the end of the chromosome has been suggested.

Conclusions: This study demonstrates that low-coverage WGS provides highly accurate, low-cost diagnosis of aneuploidy in cells from patients with congenital chromosomal abnormalities.

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P14.025A

Our Genematcher data sharing experience: 10 days on average to confirm the pathogenicity of a candidate gene

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Whole-exome sequencing (WES) has proven to be a powerful tool to identify the molecular bases of heterogeneous conditions such as intellectual disability (ID) and/or multiple congenital abnormalities (MCA). A large number of results remain non-conclusive, especially for ultra-rare conditions that limit genotype-phenotype correlations. International data-sharing was used to identify additional patients carrying variants in the same gene, in order to draw definitive conclusions on their implication in the disease. Here, we report our experience using the GeneMatcher initiative, a data-sharing platform designed to enable connections between clinicians and researchers to help solve 'unsolved' exomes and to identify new genes. Over the last two years, we have shared 70 candidate genes identified by WES performed in individuals affected by ID/MCA. We evaluated the ability to determine the involvement of these genes and the necessary timeframe: 59/70 genes (84%) were matched to at least one other mutated individual, and 23 genes recurring in additional affected individuals were identified as the probable cause of a developmental disease (39%). The waiting period between submission and the first match varied, with an overall median of 4 hours. When a match occurred, the median response time between the first email to contact a submitter and the response was estimated at 31 hours. The rapid identification of these new genes remains essential for clinical characterization, genetic counselling and for translation to the diagnostic field. GeneMatcher appears to be a very efficient tool to identify new genes in highly heterogeneous conditions.

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P14.026B

Challenging detection of a novel deletion at the exon-intron junction in *MYH11*

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Introduction: The detection of deletions comprising mainly intronic and only small exonic regions are challenging as they may be missed in exome and gene panel analysis with standard software (including copy number variation tools) and analysis settings. Here we report an analysis approach to overcome this limitation.

Patient: We investigated a 37 y old man who was currently hospitalized due to an iliac artery aneurysm. At the age of 19 y the patient had undergone surgical correction of an acute type A aortic dissection. Family history was negative.

Method: Massive parallel sequencing of 10 genes: *ACTA2, FBN1, TGFBR1, TGFBR2, TGFB2, SMAD3, COL3A1, MYH11, MYLK, TGFB3* (GRCh37, hg19); library: TruSight™One, Illumina; sequencer: NextSeq, Illumina; data analysis incl. CNV: SeqNext [JSI] and cnMOPS. Breakpoint detection by long range PCR and sequencing; RNA analysis after cell culture.

Result: At the exon-intron 29 border of *MYH11*, SeqNext called a delins variant followed by unaligned sequence in a small fraction of reads (26%). The aberrant sequence was manually aligned to a 3´ intronic sequence, representing a heterozygous deletion c.3878_3880-85del. This deletion comprises 1773 bp and include the last two bases of exon 29 and a large part of intron 29. RNA analysis is ongoing to determine the exact effect on transcript level.

Conclusions: The detection of large "mainly" intronic deletions by panel/exom sequencing is challenging as CNV-tools are not easily able to characterize them. Heightened awareness and additional methods such as RNA analysis are required for identification and confirmation of such deletions.

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P14.027C

Copy number variant detection increases diagnostic yield of Mendeliome sequencing

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Introduction: Although complicated by the fragmented nature of the next-generation targeted resequencing data, copy number variants (CNVs) may be detected by read-depth analysis. Along with whole exome sequencing, sequencing of large panels of disease-associated genes (Mendeliomes) are widely used in clinical practice. The diagnostic utility of CNV detection from Mendeliome sequencing, however, remains underreported.

Materials and Methods: TruSight One panels (Illumina Inc., San Diego, CA) targeting 4813 genes were sequenced as a diagnostic test in 1407 consecutive patients at our department. The indications for testing were not restricted to any disease group; many patients had undergone previous molecular testing including chromosomal microarrays. Raw sequencing reads were mapped to hg19 reference genome. In addition to small variants, CNVs were called using CoNIFER software. All reported CNVs were validated as appropriate.

Results: Among 1407 patients conclusive genetic diagnosis was made after Mendeliome sequencing in 327 (23.2%), accompanied by additional 10.8% patients in whom variants of unclear clinical significance were reported. Rare CNVs were reported for 30 patients. The detected CNVs ranged from single exon to contiguous gene deletions. In additional two cases, X-chromosome aneuploidies were suspected after noticing variant read ratio discrepancies. In 18 (1.3%) patients, the CNVs were classified as disease causing, while others remained of unclear significance. In 4/18 cases, the CNV was identified in trans with pathogenic small variant.

Conclusions: CNV detection improved diagnostic yield of Mendeliome sequencing by over 1% without increasing costs significantly, and thus should be encouraged in all clinical laboratories.

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P14.028D

Developing a Test Directory for Rare and Inherited Disease Genomic Testing in the National Health Service in England

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To enable the National Health Service (NHS) to fully benefit from the advances in genomics, building on its long history of laboratory and clinical genetics, NHS England is

establishing a new national Genomic Medicine Service. The service will deliver a nationally agreed repertoire of tests set out in a single, annually refreshed, 'Test Directory', covering the range of genomic testing for Rare and inherited disease and Cancer from highly targeted testing to whole genome sequencing.

We describe the structured approach used to create the Rare and inherited disease directory and the contents of the resulting directory.

With the support of experts in each area, 'Clinical indications' for testing were linked to 'Test scope' (relevant variant types and loci) and then 'Test method(s)' capable of testing across the defined scope. A previously presented evaluative framework was used to inform the optimal approach in each setting (Scott et al ASHG 2017).

The directory contains 350 clinical indications with 452 test method components including 24 indications for whole genome sequencing. It includes 61 'core' indications, covering the bulk of testing by volume, to be performed at each of the new laboratory hubs and 289 'specialised' indications to be performed by a subset.

The directory will be a component of a National Genomics Informatics System that captures all test requests and results to facilitate monitoring of activity and equity of access to testing and provide data crucial to ongoing assessment of optimal testing approaches and other quality improvement activities and, for consented patients, research.

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P14.029A

A new powerful PCR-based assay for the molecular diagnosis of myotonic dystrophy type I

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Myotonic dystrophy type 1 (DM1) is caused by expansion of the CTG repeats in the 3' UTR of *DMPK* gene. The conventional diagnostic strategy is based on a fluorescent PCR (amplification up to 110 CTG), followed by Southern Blot for apparently homozygous patients. It is therefore expensive and time-consuming. We have assessed the sensitivity and specificity of a new prototype diagnostic procedure (Asuragen's AmplideX® PCR/CE *DMPK* technology) on 49 samples (7 controls and 42 DM1-patients).

DNA were isolated from different tissues (blood, chorionic villi and amniotic fluid) and amplified by PCR (*DMPK* gene specific PCR (GS-PCR) and TP-PCR). FAM-labeled amplicons were resolved by capillary electrophoresis (CE). The AmplideX® PCR/CE assay gave expected results for the 49 samples (100% sensitivity and specificity) including resolution of homozygous and heterozygous samples, and those with large CTG expansions (57-1800 repeats). Furthermore, previously unidentified mosaicism was revealed in 6 patients.

Ten samples from DM1-patients (85-1500 repeats) were further sized by GS-PCR and agarose gel electrophoresis. In one sample, the expanded allele (1300 CTG) revealed by Southern blot analysis, was not detected.

The AmplideX® PCR/CE *DMPK* technology is therefore able to efficiently detect expanded alleles and differentiate homozygous genotypes from others. It is able to size alleles with a high resolution from 5 to 200 repeats, detect expanded alleles up to at least 1,800 repeats, and size expanded alleles up to at least 1,500 repeats. AmplideX® PCR/CE *DMPK* technology appears to be a simpler and faster approach than conventional techniques for the molecular DM1 diagnosis.

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P14.030B

Interstrand crosslinked DNA in body fluids of cancer patients treated with a platinum agent

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Introduction: Northern Lights Assay (NLA) is a versatile technique for detecting various types of DNA damage in body fluids including single-stranded breaks, double-stranded breaks, intrastrand and interstrand DNA cross-links (ICL), single-stranded DNA and bulky lesions. With NLA we tested whether specific DNA lesions in body fluids are seen in colon cancer patients treated with a combination therapy of a platinum crosslinking agent and a thymidylate synthase inhibitor.

Materials and Methods: Randomly selected 27 colon cancer patients treated with standard CapOx infusion including capecitabine and oxaliplatin (125 mg/m^2). We collected a complete set of plasma, saliva and urine from each patient immediately after the infusion, isolated DNA

and analyzed with NLA. NLA based on Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE) in microgels.

Results: We detected ICL in urinary sediments in all patients analyzed. ICL were detected in cfDNA in plasma in 7 patients and nicked, double-stranded, nucleosomal cfDNA was detected in 9 patients. Of those patients 4 had both types of DNA lesions.

Conclusions: Treatment with the platinum agent oxaliplatin commonly results in interstrand crosslink DNA in urinary sediment cells and sometimes in plasma cfDNA right after infusion. The finding of nicked, double- stranded, nucleosomal cfDNA could result from treatment with the thymidylate inhibitor capecitabine comprising de novo DNA synthesis, oxaliplatin and/or from cellular stress. These DNA lesions at different time points after therapy could be potential biomarkers in cancer theragnostics to evaluate response to treatment or risk of side-effects.

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P14.031C
DSP30 as a mitogen in B-cell malignancies including those of a low disease burden

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Chromosome abnormalities detected during cytogenetic investigations for B-cell malignancy offer prognostic information that can have wide ranging clinical impacts on patients. These impacts may include monitoring frequency, treatment type and disease staging level. The use of the synthetic oligonucleotide DSP30 combined with interleukin 2 (IL2) has been described as an effective mitotic stimulant in B-cell disorders, predominantly in chronic lymphocytic leukaemia (CLL) but also a range of other B-cell malignancies. Here we describe the comparison of two B-cell mitogens, lipopolysaccharide (LPS) and DSP30 combined with IL2 as mitogens in a range of common B-cell disorders excluding CLL. The results showed that DSP30/IL2 was an effective mitogen in mature B-cell disorders, revealing abnormal cytogenetic results in a range of B-cell malignancies. The abnormality rate increased when compared to the use of LPS to 64% (DSP30/IL2) from 14% (LPS). In a number of cases the disease burden was proportionally very low, less than 10% of white cells. In 37% of these cases, the DSP30 culture revealed abnormal results. Importantly, we also obtained abnormal conventional cytogenetics results in 3 bone marrow cases in which immunophenotyping showed an absence of an abnormal B-cell clone. In these cases the cytogenetics results correlated with the provisional diagnosis and altered their staging level. The use of DSP30 and IL2 is recommended for use in many B-cell malignancies as an effective mitogen and their use has been shown to enable successful culture of the malignant clone, even at very low levels of disease

K. Dun: None.

P14.032D
A gene-agnostic trio exome sequencing strategy outperforms gene panel analysis

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The identification of the genetic aetiology in children with non-specific/heterogeneous phenotypes, or without the full spectrum of syndromic features, poses a challenge. For these cases, selection of a gene panel for next generation sequencing (NGS) can be restrictive whereas a gene-agnostic inheritance-based whole exome trio offers a more comprehensive analysis. We analysed 266 consecutive trios referred for diagnostic testing. Whole exome sequencing was performed using Agilent (v5/6) capture and Illumina sequencing (HiSeq2500/ NextSeq500). Rare potentially deleterious variants were filtered by mode of inheritance and classified using ACMG-AMP guidelines. We ascertained whether the disease gene was included in at least one Genomics England PanelApp gene panel. Likely pathogenic/pathogenic variant(s) were identified in 43% (113/266) of cases. For 9 patients the disease gene was not included within a PanelApp gene panel and the maximal diagnostic yield for a phenotype-driven panel analysis would have been 39%. In 6 cases the genes were very recent

discoveries where our findings have provided further evidence to support the disease gene association (e.g. *KLH7*, *RHOBTB2* and *AIMP2*). We identified three strong biological candidate genes (*NXN*, *NAXD* and *PIGB*) and via GeneMatcher found additional cases/collaborations that subsequently confirmed these as novel disease genes. The gene-agnostic trio approach using inheritance filtering enabled a diagnosis for an additional 9 patients (8% of diagnoses). We will discuss how the fast pace of gene discovery in the NGS era coupled with the clinical/genetic heterogeneity provides a strong case for using this strategy combined with GeneMatcher and future re-analysis to maximise diagnostic yield.

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P14.033A

Improving the performance, cost, and flexibility of custom target enrichment & whole exome sequencing

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Whole Exome Sequencing and Target Enrichment approaches provide a powerful solution for effectively focusing sequencing costs on genomic regions of interest. However, hybridization-based enrichment can pose various challenges leading to context-specific biases and lack of uniformity affecting quality and efficiency. Here we present data obtained using Twist Bioscience's new NGS Target Enrichment System, which shows substantial improvements in multiplexing capacity with single-plex performance, high uniformity with fold 80 base penalties as low as 1.3, and near optimal capture across low to high GC content regions. Altogether, these improvements lead to a target coverage of up to >98% and >95% of bases at 20x and 30x respectively with only 4.5GB sequencing, a much wider linearly scalable cost to coverage relation for high-coverage applications, and >99% precision and sensitivity in variant calling on both hg38 and the hg19 human assemblies which are natively supported. These results are possible due to a carefully designed system including double-stranded biotinylated probes, an optimized stringency workflow, and efforts focusing on optimizing bait design and target profiles. This allows capturing 99% of potentially pathogenic clinVar variants in just 33MB of coding sequence targets based on a CCDS exome, or can be incorporated in custom user designs taking advantage of Twist's unique ability, flexibility, and fast turnaround time in high quality DNA synthesis.

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P14.034B

Rapid whole exome sequencing in critically ill children

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Introduction: We recently successfully explored rapid diagnostic whole-genome sequencing in critically ill newborns, aiming to improve their clinical care and replace time-consuming and/or invasive diagnostic testing. Now, we implemented rapid sequencing in standard diagnostics, with adapted procedures and an expanded age-range to include all critically ill children.

Materials and Methods: Twenty-five critically ill or deceased patients under the age of 16 years were included between April 2017 and February 2018.Trio exome sequencing was performed using Agilent SureSelect XT exome v6 capturing . After filtering using a virtual gene-panel of 3850 monogenic disease genes and inheritance-mode, selected variants were discussed in a multi-disciplinary team. Pathogenic or likely pathogenic variants matching the patients' phenotype were reported. Variants in genes not matching the patient's phenotype but considered as actionable, were reported as incidental findings.

Results: Turnaround time was less than 14 days in all samples. Pathogenic or likely pathogenic mutations explaining the phenotypes were detected in 9 of the 25 children (36%) and involved the *MTM1, PDHA1, MAP3K7, KCNQ2, PTPN11, KCNT1, TBCK and CRLF1* and *CHD7* genes. Incidental findings with clinical consequences were found and communicated in two families.

Conclusions: Rapid trio-exome sequencing is feasible and has a high yield (36%) and a speed comparable with whole genome sequencing with targeted panel analysis.

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P14.035C

Systematic dissection of biases in whole exome and whole genome strategies for human genome resequencing reveals major determinants of human coding sequence coverage

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Introduction: Advantages and diagnostic effectiveness of whole exome (WES) vs. whole genome (WGS) sequencing in research and clinical practice are still heavily debated. In our study, we took an effort to systematically assess coverage of CDS regions provided by several modern WES platforms and a PCR-free WGS kit.

Materials and Methods: We performed bioinformatic analysis of 312 WES samples sequenced with four technologies (Agilent SureSelect, Roche SeqCap EZ MedExome, Illumina Nextera Rapid Capture, and Illumina TruSeq Exome), as well as 17 PCR-free WGS samples.

Results: We identified the ~400 kb region of human exome that could not be effectively characterized using short (2x150) read technology and b37 human genome reference. Using several novel metrics to characterize exon coverage in WES and WGS, we showed that some of the WES platforms (SureSelect and MedExome) achieve substantially less biased CDS coverage than others, with lower within- and between-interval variation and GC-content bias. We also showed that the overall power for SNP and indel discovery in CDS region is indistinguishable for WGS and best WES platforms.

Conclusions: Our results show that deep WES (100x) with least biased technologies can provide similar coverage (97% of 10x bases) and CDS variant discovery to the standard 30x WGS. Both WES and WGS are effectively incapable of variant discovery in low mappability regions of the exome due to limitations of short read technology. Our study may help to select the resequencing approach for human genetics studies.

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P14.036D

Increased burden of possibly pathogenic variants in disease-associated genes in patients with negative exome sequencing result

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Although exome sequencing has considerably improved diagnostic yield in patients with suspected genetic diseases, it is still not possible to identify a conclusively causative genetic variant in a notable proportion of patients. In these cases, we nevertheless observe multiple residual findings in genes associated with referral phenotype, including variants of unknown significance and carriership of known pathogenic and known risk factor variants. To assess the relevance of these findings, we studied if patients with negative or inconclusive diagnostic exome result carry an excess of variants in genes associated with referral diagnosis. We selected 196 patients with negative diagnostic exome result from five broad disease groups, including hypertrophic cardiomyopathy, skeletal myopathies, hearing loss, epilepsy and Parkinson disease (PD). For each group, we systematically assessed the burden of variants in disease-associated genes and compared this rate in patients versus controls. We observed a consistent excess of known pathogenic and loss-of-function variants in disease-associated gene panel for all five surveyed disease categories. The difference was most notable in case of PD, where patients had on average 5.2 times more variants in PD-associated gene panel versus controls, followed by hypertrophic cardiomyopathy (1.7x), myopathies (1.4x), hearing loss (1.4x) and epilepsy (1.1x). In conclusion, we observed an overall excess of possibly pathogenic variants in disease-associated genes in patients with negative or inconclusive exome results. This suggests that residual findings in exome data might be relevant in these patients and further stresses the need for improvement

of variant interpretation process, especially by sharing data across multiple institutions.

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P14.037A

DeepGestalt- using deep learning to detect rare genetic syndromes from facial phenotype

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Introduction: Advances in machine-learning, computer vision and deep-learning enable computerized systems to prioritize variants through recognition of syndromic phenotypes. One of the main challenges for such a system is being able to generalize well for hundreds of (individually rare) genetic syndromes from small amounts of data.

Methods: DeepGestalt is a machine-learning framework that uses deep convolutional neural networks (DCNNs) to score genetic syndromes by analyzing frontal facial 2D-photos. The framework was trained stepwise: First, face location and facial landmarks were automatically detected, followed by face alignment and cropping into multiple regions. Then a general face representation was learned using DCNNs and used to train a specialized multiple DCNN. Results are aggregated and sorted to obtain the final ranked list of syndromes. DeepGestalt was trained on a phenotype-genotype database curated by clinical geneticists using a community-driven tool called Face2Gene (FDNA Inc, USA) and is the framework powering this tool.

Results: 1. Multiclass: 502 images of randomly sampled diagnosed cases, yielded 91% top-10-accuracy for 216 different syndromes. 2. Noonan genes: Clinicians and DeepGestalt were asked to classify photos of patients diagnosed with any of 5 gene-mutations in Noonan Syndrome, yielding no recognition by clinicians while DeepGestalt had 64%-top-1-accuracy, compared to a 20%-random chance. 3. Yes/No type testing recognizing Angelman Syndrome, yielding 71%-accuracy by clinicians while DeepGestalt identified 92%.

Conclusions: We suggest that this form of artificial intelligence is ready to support medical genetics in clinical and laboratory practices and will play a key role in the future of precision medicine.

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P14.038B

Extraction and sequencing of DNA from human tissue fixed and stored in formalin

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Introduction: Formalin is widely used to preserve human tissues for pathological investigation. However, the DNA isolated is usually fragmented and corrupted, limiting its use for diagnosis or research. We wanted to extract high-quality DNA from formalin-fixed tissue suitable for use in Sanger and next-generation exome sequencing.

Methods: Using human tissue stored in formalin for 12 months, we created a novel protocol to extract high-quality DNA, combining proteinase-K and heating with Chelex resin. We also evaluated the effect of uracil-DNA-glycosylase (UDG), an enzyme suggested to remove formalin artefacts. The yield, purity, fragment size distribution, efficacy as a template for PCR and sequences of PCR products were compared. Using this protocol, next-generation whole exome sequencing was performed on DNA extracted from an explanted heart that had been stored in formalin for over two years. The results were compared with those from freshly obtained DNA (blood) and the utility of Sanger sequencing as a confirmatory strategy was also evaluated.

Results and Conclusions: High-quality DNA was obtained from formalin-fixed tissue using our protocol. This increased the PCR product length obtained from 200 to

400bp. However, whilst UDG removed some artefacts it introduced others. Results of NGS exome sequencing on formalin-fixed DNA compared favourably to fresh DNA with 94% specificity, but 73% sensitivity. Thus, it is possible to obtain high-quality DNA template from formalin-fixed samples, but limited sensitivity to detect all variants limits its use to confirmation of known, rather than discovery of novel, variants.Supported by Saudi Arabia Cultural Bureau and Ministry of Education

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P14.039C

Generalization of an Assay System using Repeat-primed PCR and Capillary Electrophoresis to Resolve Multiple AT- and GC-rich Short Tandem Repeats Associated with Neurological Diseases

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Introduction: DNA repeat sequences constitute roughly 50% of the human genome. Short tandem repeats (STRs) impact human disease through variable length effects on gene expression, splicing, and translation. Over 30 Mendelian disorders are associated with STR expansions, yet diagnostic applications have often been thwarted by a lack of rapid, simple, and accurate assay systems. Here we describe the analytical performance of multiple repeat-primed (RP) PCR assays* resolved on the SeqStudio Genetic Analyzer.

Materials and Methods: Mono-, tri-, and hexa- nucleotide repeat expansions from AT- and GC-rich STR disease markers (*TOMM40, FMR1* and *C9orf72*) were amplified from clinically-derived samples (n=49) using AmplideX® PCR/CE kits* (Asuragen). FAM-labeled amplicons were resolved on SeqStudio and 3500 Genetic Analyzers (Thermo Fisher).

Results: All genotype results were concordant between instruments and deviations were at most one repeat within the sizeable range. Tunable run conditions extended repeat quantification by 25% (to 180 *C9orf72* G_4C_2 repeats) from baseline conditions, enhanced expanded peak detection sensitivity by up to 100-fold, and enabled rapid run times as short as 20 min. Further, different assays could be analyzed on the same plate to improve batch run efficiency. Studies are ongoing to assess STRs in myotonic dystrophy type 1 and Huntington's disease.

Conclusions: We show that single-tube, long-range RP-PCR can be adapted to different repeat sequences using high-resolution, easy-to-use fragment sizing platforms. This flexibility may accelerate the development and adoption of STR-based assays for clinical research and molecular diagnostics.

*For Research Use Only, Not for Use in Diagnostic Procedures

G.J. Latham: A. Employment (full or part-time); Significant; Asuragen, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Asuragen, Inc. **C. Redmond:** A. Employment (full or part-time); Significant; Asuragen, Inc. **K. Nicholson:** A. Employment (full or part-time); Significant; Asuragen, Inc. **E. Lagow:** A. Employment (full or part-time); Significant; Asuragen, Inc. **J. Wallace:** A. Employment (full or part-time); Significant; Thermo Fisher, Inc. **E. Schreiber:** A. Employment (full or part-time); Significant; Thermo Fisher, Inc. **S. Higdon:** A. Employment (full or part-time); Significant; Thermo Fisher, Inc. **E. Bram:** A. Employment (full or part-time); Significant; Asuragen, Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Asuragen, Inc.

P14.040D

Impact of improving gene annotation on diagnostic yield of Mendelian disorders

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Genetic diagnosis of Mendelian disorders requires clinical scientists to distinguish pathogenic variants from many potentially functional variants present in any human genome. Variant interpretation is reliant on gene annotation; however, as our understanding of transcriptomic complexity improves it is apparent that existing annotation is incomplete. Comparison of Ensembl and AceView annotations reveals over 15,800 genes that lie in intronic or intergenic regions according to the other database. Consequently, incorrect or incomplete annotation may cause pathogenic variants to be misassigned, hidden amongst false positives or overlooked within mis-annotated non-coding regions. To address this problem, we used publicly available transcriptomic data to improve the annotation of 2806 OMIM-morbid genes. We obtained annotation-agnostic quantification of transcription from 54 GTEx tissues using recount2 and combined this with RNA-seq reads spanning exon-exon junctions to link putatively transcribed regions to known

OMIM genes. We characterised these transcribed regions using sequence properties (leveraging information from ENCODE and using ORFfinder) and their expression in relation to the reference gene. Despite OMIM genes having been extensively studied, we discovered over 5Mb of additional unannotated transcription within 50Kb of an OMIM gene, which predominantly fell within intronic regions (72%). Restricting our analysis to non-overlapping genes, we find that 0.35Mb of transcribed regions connect to known OMIM isoforms, many of which are expressed within disease relevant tissues. Overall, we improve the annotation of OMIM genes, a vital step for the accurate assignment of variant pathogenicity, and anticipate that this will lead to improvements in diagnostic yield from whole genome sequencing.

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P14.041A

Rapid genetic diagnosis employing whole exome sequencing for critical illness in infants and children

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Critical illness in infants and children is one of the most challenging fields in maternal and children health. Because precise managements in respective to the diseases are often not possible in patients with genetic diseases, the prognosis can be poor even though a large amount of resources are consumed. This project employs rapid whole exome sequencing (WES) and artificial intelligence-assisted data processing to fit the needs of acute disease managements. The inclusion criteria include patients admitted to intensive care unit and suspecting to have genetic etiologies, and/or inborn error of metabolism. A familial trio were sampled for WES analysis. From May 2017 to Dec 2017, 23 patients were enrolled for WES trio analysis. The clinical diagnosis of enrollment includes shock (n=4), seizure (n=7), Respiratory failure (n=5), Sudden infant death syndrome (n=1), Abnormal newborn screening (n=2), and Undiagnosed disease with multiple systemic involvement (n=4). Of them, 13 (56.5%) had molecular diagnosis. The mean duration of turnaround time to have tentative diagnosis is was 6.3±1.4 days (range 4.3-9.9 days). Disease causing genes identified included Immunodeficiency (n=2; *CD3E* and *C3*), Cardiomyopathy (n=2; 2 *COQ4* cases), Channopathy (n=3; *KCNQ5*, *RYR2*, and *SCN8A*), Peroxisomal biogenesis disorder (n=2, *PEX1* and *PEX7*), congenital myopathy (n=1, *ISPD*), Myelination disorder (n=1, *EIF2B5*), congenital disorder of deglycosylation (n=1, *NGLY1*), and Axenfeld-Rieger syndrome (n=1, *PITX2*). Therefore, in this study, we demonstrated the possibility of shortening the diagnosis duration of WES for ICU patients with potentially genetic disease. This project is funded by the Mistry of Health and Welfare.

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P14.042B

A simple and fast method for direct-to-PCR genotyping

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Introduction: PCR is known to work well on crude and complex templates unless interfering substances are present. In case of blood, it is essential to remove heme, which is a potent inhibitor of most DNA polymerases.

Material and Methods: We have developed a simple, inexpensive and time-saving method to skip DNA extraction when using Taqman-based ViennaLab RealFast assays for genotyping and variant detection on fresh and frozen blood. After a 10 min heat denaturation and quick centrifugation step, the resulting supernatant is diluted into direct-to-PCR (D2PCR) buffer and directly used as template in PCR.

Results: The D2PCR approach for RealFast assays can be performed on any common qPCR instrument capable of detecting FAM/HEX (single-plex assays) or FAM/HEX/ROX/Cy5 (duplex assays) fluorescence. Optimal time saving is achieved only when combining D2PCR testing with fast mode thermocycling on the Magnetic Induction Cycler (MIC; Bio Molecular System). Using magnetic induction technology for heating and fan forced air for cooling, 40 cycle PCR runs can be shortened to 40 min, and the analysis of samples from drawing blood to final result can be completed in less than one hour.

Conclusions: Reduction of the turnaround, and in particular the hands-on time for molecular genetic assays is key to increasing the capacities and daily throughput of a diagnostic laboratory. As a consequence, costs can be reduced and delivering reports to patients can be accelerated.

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P14.043C

Integrated functional characterization of a non-coding *GPC3* allele in a family with recurrent Simpson-Golabi-Behmel syndrome

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Sipson-Golabi-Behmel syndrome type 1 (SGBS1 -MIM 312870) is an X-linked condition occurring primarily in males, characterized by pre- and postnatal overgrowth, coarse facial features, variable intellectual disability, congenital heart defects, supernumerary nipples, hepatomegaly and increased risk to develop neoplasia such as Wilms tumor. SGBS1 is usually caused by deletion or mutation in the gene encoding glypican-3 (*GPC3*), an outer membrane glycoprotein implicated in WNTs, Hedgehogs (Hh), fibroblast growth factors (FGF), and bone morphogenetic proteins (BMP) signaling modulation. To identify the genetic variant responsible for recurrent cases of Simpson-Golabi-Behmel syndrome type 1 within a family with negative whole exome sequencing (WES) results, we performed an integrated genomic and transcriptional analysis. Whole genome sequencing (WGS) coupled with patient-derived fibroblasts RNA expression analysis was performed in order to interrogate non-coding regulatory mechanisms, potentially accounting for dysregulation of *GPC3*, *GPC4*, *CXORF5* or other candidate genes implicated in overgrowth syndromes for a differential analysis. WGS allowed us to identify the presence of a complex rearrangement resulting in a duplication of Xq24 with concomitant deletion in Xq26.2 corresponding to a portion of the second intron of the gene *GPC3*. RNA expression not only revealed *GPC3* loss of expression, but also allowed the identification of SGBS-specific transcriptional pathways associated with glypican-3 knockout. We provide evidence of the utility of genome-wide genetic and expression analyses as complementary approaches to interrogate the transcriptional readout of non-coding variants in an ultra-rare genetic condition with clear clinical delineation and negative or inconclusive routine laboratory results.

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P14.044D

Widespread uptake of the Human Phenotype Ontology (HPO) in the National Health Service (NHS) in England as part of the 100,000 Genomes Project

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The 100,000 Genomes Project is an NHS transformation project which is founding a national research database linking genomic and clinical data from thousands of patients with rare disease or cancer. The rare disease programme recruits individuals across >200 recruitment categories, spanning the majority of monogenic diseases. Phenotype data have been collected primarily as HPO terms. For each recruitment category, a 'questionnaire' of relevant HPO terms (1 to 82 per category, median 26.5) is presented to clinicians in an electronic data capture tool, with the facility to add extra terms where relevant. Phenotypic data have been collected on >20,000 participants with a mean of 6 positive and 20 negative terms from around 1,000 contributing clinicians. HPO terms have directed application of gene panels beyond the recruited disorder, with 24% of diagnostic variants lying within these additional panels. On review of the data, 2% of families needed further clarification or enrichment of the terms initially entered to inform the analysis. HPO functions better in some clinical contexts (e.g. paediatric developmental disorders) than in others (e.g. inherited cancer, due to limited capture of histological subtypes and precise age of onset). Using set HPO questionnaires standardises the pattern of data capture between users and encourages depth of capture of positive and negative features; however, this approach may limit the capture of additional relevant phenotypes and introduce bias into the dataset. Our experience indicates that HPO is an intuitive and rich ontology which can be used at scale in frontline healthcare.

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P14.045A

Molecular Diagnosis of Huntington Disease using Nanopore Sequencing

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Detection of repeat expansions is challenging for short read next generation sequencing and fluorescent PCR assays are limited by their inability to amplify large expansion alleles. Long read sequencing technology such as Oxford Nanopore Technology (ONT) has the potential to overcome these limitations. We investigated the performance of ONT sequencing in Huntington Disease using a 2.1kb amplicon that includes the unstable pathogenic CAG and polymorphic CCG repeats. 48 samples tested by fluorescent PCR spanning 15 to 101 CAG repeats (including all alleles in the intermediate and reduced penetrance ranges) were analysed. Amplicons were prepared using the Nanopore 1D barcoding protocol and analysed on MinION Flow Cell (R9.4). FASTQ files were generated using Albacore (ONT) to perform both basecalling and demultiplexing. BAM files were generated using minimap2. Allele calls were performed by RepeatHMM* (with the predefined HTT model) using FASTQ and BAM inputs. Repeat length estimates by both Nanopore sequencing and fluorescent PCR showed a concordance (R^2) >0.9957. RepeatHMM analysis with BAM inputs produced the best results with a specificity of 0-1 repeat for alleles with 20-39 repeats. However, alleles with <20 repeats were overestimated by 1-2 repeats. The 2.1kb amplicon reduced the preferential amplification of the smaller alleles in relation to large expansion alleles, thereby increasing the specificity of the assay. Provided sequencing depth was 100-fold or greater, we achieved high accuracy of repeat calling. This study shows that Nanopore long-read sequencing has the potential to provide a diagnostic assay for repeat expansions. *Lui et al. *Genome Medicine (*2017) 9:65

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P14.046B

The impact of reference panel short-read sequencing inaccessibility on genotype imputation

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Imputation is now a routine step in genome-wide association studies (GWASs) and has facilitated the discovery of many novel signals. While the validity of genetic imputation algorithms has been determined, the limitations to imputation reference panels from short-read sequencing inaccessibility has not been systematically assessed. Here, we investigated these limitations and their impact on GWAS in the Cooperative Health Research In South Tyrol (CHRIS) study, a population based study with 4,661 genotyped and phenotyped individuals. By comparing imputed genotypes to microarray genotypes in the CHRIS study, we demonstrated a low quality of imputation for variants present in regions defined by the 1000 Genomes Project Phase 3 as inaccessible to short-read sequencing. Screening the summary statistics of 58 biochemical trait GWASs performed in the CHRIS study, we detected an aspartate aminotransferase associated locus in an inaccessible region that is driven by microarray genotyped variants, and cannot be reproduced through 1000 Genomes imputation. Consistently, in the publicly available NHGRI-EBI GWAS catalog and in UK Biobank GWASs, we observed a lower density of trait-associated variants in inaccessible regions when compared to accessible regions. In summary, we show that the nature of short-read sequencing affects the ability of GWASs to discover trait associated loci in inaccessible regions.

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P14.047C

X-chromosomal INDELs examined by ddPCR for female sex determination in NIPD and in forensic science

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Introduction: In families with gonosomal recessive diseases, fetal sex is determined prenatally by detection of Y-chromosomal sequences in cell-free fetal DNA in maternal plasma. When these sequences are not found, female sex of the fetus is reported. Here, we present a methodology allowing the detection of paternal X-chromosomal alleles on maternal background and the confirmation of female sex by positive amplification signals.

Material and Methods: Using ddPCR we examined X-chromosomal INDELs: rs2307932, rs16397, rs16637, rs3048996, rs16680 in buccal swabs of 50 females to receive the population data. For all examined INDELs, we tested the performance of ddPCR for mixtures 20%, 10%,

5% and 2.5% of one homozygote on the background of the opposite one. We examined the cell-free plasma DNA from 13 pregnant women bearing Y-chromosome negative fetuses.

Results: We detected the minor fraction (representing the paternal X-chromosomal allele on the maternal background) in all artificial mixtures. We confirmed the presence of paternal X-chromosome in 12 out of 13 female bearing pregnancies (92.31% sensitivity).

Conclusions: We developed the ddPCR approach allowing the determination of paternal X-chromosomal alleles on maternal background for non-invasive prenatal diagnostics. This approach may be used for prenatal paternity exclusion and for determination of female sex in forensic samples. Supported by grants no. VI20172020102 by Ministry of Interior of the Czech Republic, no. Progres Q25/LF1 and no. SVV 260 263 of the Ministry of Education, Youth and Sport of the Czech Republic, and by the grant RVO-VFN 64165 of the Ministry of Health of the Czech Republic.

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P14.048D

A novel duplex-ready library construction method for improved conversion of low-input and highly degraded DNA

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Diagnostic tools based on next generation sequencing (NGS) are fundamentally transforming clinical oncology. Current library preparation strategies only allow relatively low library conversion, especially when the input is low, making the detection of low-frequency mutations by NGS particularly challenging. In addition, mutation artifacts arise from various sample storage and preparation processes, which can significantly lower the positive predictive value (PPV) of a clinical NGS diagnostic assay. These artifacts can be identified by "duplex sequencing", where strand-specific unique molecular identifiers (UMIs) are used to filter out artefactual DNA damages. The duplex sequencing strategy requires high conversion in library preparation to enable sequencing and pairing both strands of the same DNA molecule.

To achieve high library conversion for low input DNA samples and to increase sensitivity and PPV, we developed a novel library construction chemistry. The method provides superior library conversion efficiency using a unique, mutant DNA ligase and sequencing adapters that increase ligation efficiency and suppress chimera formation as well as adaptor dimers. We tested the performance of our method using three sample types: sheared genomic DNA, cfDNA, and FFPE DNA. We also tested its performance in liquid biopsy applications using libraries from mixtures of NA12878 and NA24385 cell line DNA with mutant allele fractions (MAFs) down to 0.2%. When compared to commercially available methods, our approach yielded significant increase in sensitivity and PPV, especially in low input range (1-25ng). Our results demonstrate that our methodology provides a useful tool for applications where high conversion of input DNA samples is needed.

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P14.049A

High-throughput SMRTSequencing of clinically relevant targets

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High throughput sequencing of targeted genomic DNA or cDNA to access disease associated causal variants using NGS approaches is a standard study practice in translation research. However, short-read data is prone to mis-mapping and coverage bias posing many challenges during variant analysis beyond simple SNPs. We explore the use of long-reads delivered by SMRT Sequencing for complete characterization of a range of genomic variants including structural variations, haplotype phasing, low-frequency SNPs, repeat expansions and GC-rich promoter regions.

We have developed flexible multiplexing options as well as analysis pipelines using Circular Consensus Sequencing

(CCS) or Long Amplicon Analysis (LAA) tools to support the targeted SMRT Sequencing applications. CCS generates a highly accurate (QV30) consensus read from each single intra-molecular multi-pass polymerase read. The CCS approach is highly reliable for identification of minor variants with allelic frequencies as low as 1 %. On the other hand, Long Amplicon Analysis (LAA) derives highly accurate consensus reads (>QV50) by *de novo* clustering subreads originating from multiple copies of the targets. LAA generates phased, full-length consensus sequences for various genes in a single sequencing run and is most useful for imputation free allele segregation.

In this work, we demonstrate SMRT Sequencing workflows for efficient and cost-effective sequencing of a broad range of clinically relevant targets from 250 bp to >10 kb. Specifically, we illustrate the advantage of SMRT Sequencing to overcome the challenges of traditional methods when genotyping *CYP2D6* and *CYP2D7* as well as high resolution four field HLA typing.

S. Ranade: A. Employment (full or part-time); Significant; Pacific Biosciences. **L.A. Aro:** A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences. **I. McLaughlin:** A. Employment (full or part-time); Significant; Pacific Biosciences. **C. Heiner:** A. Employment (full or part-time); Significant; Pacific Biosciences. **P. Baybayan:** A. Employment (full or part-time); Significant; Pacific Biosciences. **A. Toepfer:** A. Employment (full or part-time); Significant; Pacific Biosciences. **B. Bowman:** A. Employment (full or part-time); Significant; Pacific Biosciences. **R. Hall:** A. Employment (full or part-time); Significant; Pacific Biosciences.

P14.050B
Long-read sequencing goes clinical

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Next generation sequencing has revolutionized the field of human genetics by offering new possibilities to unravel human diseases. Due to limitations of short reads however, various traditional tests including single gene sequencing, MLPA or genescan are still performed, leading to a complex landscape of different available genetic tests running on a variety of platforms. A generic 'one test fits all' strategy would be a more efficient and cost-effective way to perform genetic testing. Long-read sequencing does provide an opportunity to move a step closer towards this objective. To get a feel for both platform and underlying chemistry we started with amplicon-based sequencing on the Sequel (PacBio). We sequenced amplicons up to 16kb, and learned that long-read sequencing works well for HLA typing, mtDNA, or long range amplicons designed to avoid pseudogene regions. We also reasoned that small amplicons (<1kb) can be sequenced accurately on a long-read sequencer and started to transfer our amplicon-based workflows from the IonTorrent towards the Sequel. Ultimately, we aim to combine all different workflows into one amplicon-based sequencing run. A LIMS-based automated workflow and an automated bioinformatic pipeline thereby facilitate streamlined sample processing and data analysis, and assure highest flexibility. We believe that long-read amplicon sequencing is a first step to make use of the advantages of long reads in NGS-based diagnostics, thereby allowing to combine different sequencing approaches in one test. Once the price for (long-read) genomes is at a range acceptable for routine diagnostics, targeted sequencing approaches will ultimately be replaced by genome sequencing.

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P14.051C
Standard Sanger re-sequencing protocols achieve 5% limit of detection for single nucleotide polymorphisms and insertion and deletion variants using a novel signal processing algorithm

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Introduction: Detecting minor genetic variants is essential to cancer and infectious disease management. Many have turned to next generation sequencing for this based on an assumption that the limit of detection for Sanger sequencing is a variant allele frequency (VAF) of 20%. We have recently developed algorithmic methods to reduce this to 5% for single nucleotide polymorphisms (SNPs) and insertion/deletion variants (indels).

Materials and Methods: Samples with indels spanned 33 different amplicons from 21 different genes; SNPs spanned 22 different amplicons from eight different genes. Samples were from DNA reference standards, genomic DNA, and DNA extracted from formalin-fixed, paraffin-embedded tissues. DNA was quantified using the RNase-P quantitative polymerase chain reaction assay, and serially diluted. Allelic proportions spanned 0.6125% to 50% for

SNPs and 2.5% to 50% for indels. Samples were amplified, sequenced, and pre-processed using standard protocols and tools for Sanger sequencing from Applied Biosystems™.

Results: 121 samples had deletions from 1 to 48 base pairs (bp) and 82 samples had insertions from 1 to 6-bp. 1130 samples contained 0 to 16 SNP variants. For SNPs, a detection sensitivity of 95.9% and specificity of 99.8% was observed at 5% VAF. For indel detection, there were zero false positives and zero false negatives down to 2.5% VAF. For indel characterization accuracy, type was 100%, length 100%, location 93%, sequence 93%, and VAF 92% within 3% of the expected value.

Conclusions: With new algorithms to process data from Sanger sequencing, it is possible to reliably and automatically detect 5% minor variants.

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P14.052D
Targeted sequencing analysis of commonly mutated genes in myelodysplastic syndromes using NGS: Impact and clinical implications in a single center

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The pathogenetic role of mutations in myelodysplastic syndromes (MDS) is presently investigated, and important clinical implications of these mutations are apparent. Clinical guidelines suggest including mutation evaluation in current practice. We sequenced a panel of 22 genes using Haloplex system and the Miseq platform to assess the mutational landscape of MDS patients. We evaluated 98 sequential MDS patients diagnosed in our center. At least one genomic alteration was observed in 89% of patients. The most frequently mutated genes were *TET2* (26%), *SF3B1* (24%), *DNMT3A* (22%), *SRSF2* (21%), *ASXL1* (21%), *RUNX1* (9%), and *TP53* (7%). The univariate analysis showed that only *RUNX1* mutations were associated with a shorter OS (49 months (WT) vs 12 months (mut); $P = 0.001$). We also analysed the impact of mutations in patients with normal karyotype (n = 73) where *DNMT3A* mutations conferred shorter OS (49 months vs 23 months; $P = 0.02$). A significant difference in OS was observed when two groups: OS of pts with ≥2 mutations was 25 months vs 49 months; $P = 0.003$), consistent with what shown previously. Our experience confirms that the presence of even isolated *RUNX1* mutations, although rare, identify patients with shorter survival. Patients with normal karyotype when carrying *DNMT3A* mutations or >2 somatic mutations have a significantly shorter survival than predicted by IPSS-R. Our observations, although carried out in a limited group of cases, strongly confirm the prognostic importance of mutations, and support the implementation of NGS analysis of somatic mutation, especially for MDS patients without cytogenetic abnormalities.

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P14.053A
A versatile and highly sensitive method for microRNA detection with real-time TaqMan® assays

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MicroRNAs (miRNAs) are a class of small non-coding RNAs of approximately 22 nucleotides transcribed from genomes of plants, animals, and viruses. They are highly conserved and are thought to be micro-managers of gene regulation, controlling at least one-third of human genes, therefore having a profound impact on almost every cellular pathway. Publications showing the diverse function of miRNAs have continued to increase significantly: miRNAs are involved in the developmental stages of cells, natural cell death, and major diseases such as cancer, diabetes, and cholesterol biosynthesis. MiRNAs have also been shown to be important biomarkers. Expression of miRNAs is the key step to understanding their diverse functions. We have developed a method for the detection and quantification of miRNAs that is highly specific and sensitive. By extending the miRNAs on both the 5' and 3' ends via single strand ligation and poly A tailing with universal reverse transcription, respectively, we were able to incorporate 1) unbiased pre-amplification utilizing the universal adaptor sequences and 2) flexible assay design to provide the most optimal and versatile miRNA detection. The universality of the template library from the upstream chemistry allows downstream real-time TaqMan qPCR miRNA-specific detection requiring only 1 to 10 ng of input sample. This

versatile approach allows detection of a large number of miRNAs for profiling or detection of a smaller set of miRNAs for screening and validation using the same universal cDNA template. Data for differential expression between normal brain tissue and the various cancer cell lines will be presented.

L. Wong: A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **C. Liu:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **F. Hu:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **S. Dong:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific.

P14.054B

Parental origin of deletions and duplications; about the necessity to check for cryptic inversions

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Copy number variants (CNVs) are the genetic bases for microdeletion/microduplication syndromes (MMSs). Couples with an affected child and desire to have further children are tested for a potential parental origin of the CNV routinely either by molecular karyotyping or two color fluorescence in situ hybridization (FISH), yet. In the latter case a critical region probe (CRP) is combined with a control probe for identification of the involved chromosome. However, CNVs can also appear due to other reasons, like a recombination of a submicroscopic, cryptic inversion in one of the parents. 74 patients with different MMSs and overall 81 CNVs were studied here by a novel three color FISH. The way how three locus-specific probes are selected (one in the CRP and two flanking it in a distance of 5-10 Mb) enables to detect or exclude two possible parental conditions as origins of the CNV in the index: (i) direct parental origin of the CNV (deletion or duplication) or (ii) a parental cryptic inversion. Thus, for overall 51/81 CNVs (63%) a parental origin could be determined. 36/51 (70.5%) inherited the CNV directly from one of the parents, but 15/51 (29.5%) were due to a detectable parental inversion by three color FISH. A 2:1 ratio of maternal versus paternal inheritance was found. The new, here suggested three color FISH approach increased the detection rate for parental origin in this study by 140%. Still, for 30/81 cases (37%) no reason for the 'de novo' MMS in the affected index patient could be found.

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P14.055C

Detection of 17 targets in a single PCR tube by a novel probe system combining melting curves and Taqman probes

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In many clinical settings, achieving multiplex answers from the same sample provide benefits both in terms of cost, speed, added clinical value as well as preservation of limited samples. In cases such as cancer susceptibility testing, sepsis, RSV-testing, gastrointestinal testing and many others, a broad spectrum of targets are relevant for testing. However, PCR readout is commonly limited to the current maximum of 4-5 fluorophores on most instruments. We have developed a homogenous assay method to allow readout of more than 20 answers from a single PCR reaction.

Methods: The method utilizes a system of target-specific labelled probes allowing each to be read out by subsequent melting curve analysis, allowing more than 5 probes per fluorophore channel, thereby greatly increasing the level of multiplexing achievable. By utilizing meltcurve readout of modified probes - one for each target - rather than only of the PCR amplicons, the system also adds an extra level of specificity to meltcurve analysis.

Result: To test the system, we designed probes based on previously designed TaqMan probes targeting 17 different hemorrhagic fever viruses and tested them against artificial DNA targets. Testing was perfomed in single wells containing all probes labeled with different flourophors and was able to separately detect all 17 targets. PCR was performed on a Bio-Rad CFX instrument, a MIC PCR cycler as well as the Agilent AriaMX.

Conclusions: The method comprise a robust, high-multiplex, homogeneous system to provide 20+ readouts per PCR reaction on most PCR platforms in use in current clinical diagnostics

S.M. Echwald: A. Employment (full or part-time); Significant; Anapa Biotech A/S.

P14.056D

Illuminizing the AmpliSeq™ Assay

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A challenge for any multiplex PCR assay is combining many amplicons in one reaction and achieving high target specificity. AmpliSeq™ biochemistry is the industry leading assay that allows for efficient amplification of a high number of amplicons, enabling high specificity and uniformity of coverage across a wide range of targets. We modified the AmpliSeq™ library preparation workflow so that the resulting amplicon libraries are amenable to sequencing on Illumina platforms. We designed specific dual index adapter sequences to ensure optimal performance and demultiplexing on Illumina® sequencing systems (MiSeq™, MiniSeq™, NextSeq™, iSeq™, HiSeq™) and to allow pooling of up to 96 samples into one sequencing run. We tested multiple cancer specific DNA panels as well as the Exome panel with this new workflow and investigated sequencing performance, as well as somatic and germline variant detection sensitivity and specificity on a wide variety of samples, including formalin-treated and degraded FFPE tissue samples. Further, we assessed detection of known gene fusions and evaluated gene expression measurements with RNA-specific panels. Custom panels were designed and tested over a wide range of amplicons. We observed high uniformity of coverage for all panels tested with high correlation between Illumina's sequencing platforms. Single nucleotide variants (SNVs) were reliably detected at 5% and down to 1%, even in very degraded FFPE samples with only 10 ng DNA input. Detection of CNVs was demonstrated with the Comprehensive v3 and Focus panels. We assessed variant call sensitivity and false positive rate in Platinum genome NA12878 and results will be discussed.

T. Singer: A. Employment (full or part-time); Modest; Illumina is employer. **P. Capek:** A. Employment (full or part-time); Modest; Illumina is employer. **E.S. Allen:** A. Employment (full or part-time); Modest; Illumina is employer. **T. Ghosh:** A. Employment (full or part-time); Modest; Illumina is employer. **P. Taylor:** None. **S.B. Greene:** A. Employment (full or part-time); Modest; Illumina is employer. **K. Bojanovic Machado:** A. Employment (full or part-time); Modest; Illumina is employer. **M. Chu:** A. Employment (full or part-time); Modest; Illumina is employer. **Y. Sun:** A. Employment (full or part-time); Modest; Illumina is employer. **S. Lee:** A. Employment (full or part-time); Modest; Illumina is employer. **G.J. Bean:** A. Employment (full or part-time); Modest; Illumina is employer. **I. Khrebtukova:** A. Employment (full or part-time); Modest; Illumina is employer. **J. Bruand:** A. Employment (full or part-time); Modest; Illumina is employer. **D.M. Agius:** A. Employment (full or part-time); Modest; Illumina is employer. **R.M. Kelley:** A. Employment (full or part-time); Modest; Illumina is employer. **G.P. Schroth:** A. Employment (full or part-time); Modest; Illumina is employer.

P14.057A
Improved indices for high fidelity de-multiplexing on Illumina instruments

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Demand for improved indexing capabilities has increased due to higher sequencing output, increased multiplexing to lower cost, and to avoid misassignment of reads from indexing errors. Sequencing reads may be misassigned due to "index hopping" on Illumina patterned flow cells. This misassignment can lead to false positives in ultra-sensitive variant detection, which is detrimental to analysis of liquid biopsy results and detection of low frequency (<1%) alleles. It is also necessary to eliminate errors associated with insufficient edit distances between index sequences. We have observed misassignment due to insufficient edit distance among Illumina TruSeq HT indices at a frequency up to 1.5%. Therefore, we developed 96 single (i7) 8 base indices, which can be paired with the TruSeq HT i5 indices to achieve 768-plex high throughput dual combinations. These indices were validated using a novel method on both Illumina's 2 and 4-channel technologies. This method involved the use of 96 libraries with unique, non-overlapping inserts to facilitate tracking of index misassignment. This allowed us to assess not only which index has misassigned library molecules, but to also pinpoint the origin and rate of misassignment. With our 96 i7 indices, misassignment was observed at rates <0.1%. For even higher fidelity de-multiplexing, we have paired our 96 indices as single use in both the i5 and i7 positions, known as Unique Dual Indices (UDIs). We are validating the 96 new indices as UDIs for avoidance of index hopping, and for eliminating PCR-induced chimerism during multiplexed library amplification performed during hybridization capture workflows.

L. Kurihara: A. Employment (full or part-time); Significant; Swift Biosciences. **J. RoseFigura:** A. Employment (full or part-time); Significant; Swift Biosciences. **S. Sandhu:** A. Employment (full or part-time); Significant; Swift Biosciences. **B. Lahann:** A. Employment (full or part-time); Significant; Swift Biosciences. **J. Irish:** A. Employment (full or part-time); Significant; Swift

Biosciences. **V. Makarov:** A. Employment (full or part-time); Significant; Swift Biosciences.

P14.058B

CFTR Mutation detection from newborn dry blood spots by using NGS technology

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Cystic Fibrosis (CF) is the most common life threatening autosomal recessive disease in Caucasian population and it is caused by mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene. Since an early diagnosis is known to improve the outcome of CF patients, a newborn screening (NBS) has been implemented in many countries with different strategies. In Tuscany, molecular analysis of selected CFTR variants has been introduced into NBS since 2011 for those newborns with an elevate immunoreactive trypsinogen (IRT) concentration on dried blood spots (DBSs). To improve the screening procedure, a Next Generation Sequencing (NGS) assay able to detect a panel of 276 CF-causing variants, according to CFTR2 project, has been introduced. The aforementioned NGS approach has proven to be a reliable and fast method for the identification of the CFTR variants on DNA from DBS. So far, 180 samples have been analyzed: 155 cases were negative, one variant has been detected in 22 cases whereas two variants have been identified in 3 cases. Among the identified variants, one would not have been detected by the previously used panel. The opportunity to test a larger number of variants could increase the chance to identify two CF-causing variants improving the clinical management of newborns. Furthermore, according to clinical indications, the re-analysis of NGS data concerning the whole gene sequence could be performed improving turnaround time and reducing time-consuming procedures.

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P14.059C

Application of next generation sequencing in the diagnosis of hereditary connective tissue disorders

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Introduction: The identification of a causative mutation in hereditary connective tissue disorders (HCTD) enables correct follow-up and treatment of patients and relatives. Compared to conventional methods, next generation sequencing (NGS) is expected to increase the rate of molecular findings. The pretest likelihood of findings may depend on the clinical indication as well as on the requisitioning physician.

Materials and Methods: Results from 100 exome-based analyses using a panel of 34 HCTD genes were compared to information in medical records and requisition forms.

Results: In 51 % of patients, Sanger sequencing of selected genes had previously been performed. Gene panel analysis identified a clinically relevant and likely pathogenic sequence variant in 18 % and a relevant, unclassified variant (VUS) in 10 % of samples.

A likely pathogenic variant or VUS was identified in 28 (82.4%) of genes in the panel. Likely pathogenic variants were identified in nine genes (26.5%), especially genes associated with Loeys-Dietz syndrome, all were requested from a university hospital. Requisitions indicating cardiovascular symptoms were associated with higher rate of pathogenic findings than other requisitions (19.6 % vs 6.8 %). Certain clinical symptoms were frequently noted in medical records but infrequently in requisitions.

Interpretation: Many relevant and likely pathogenic sequence variants were identified, however in HCTD the findings of VUS remains a significant challenge. A multigene panel as first tier test would reduce costs compared to Sanger sequencing preceding analysis. The likelihood of identifying a pathogenic sequence variant depended on the requisitioning instance and indication for analysis.

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P14.060D

Implementation of a new automated sample quality control tool in a whole exome sequencing workflow

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Objectives: The High Throughput Sequencing Unit of the DKFZ Genomics and Proteomics Core Facility provides general sequencing services. This project demonstrates the use of an automated electrophoresis system as a quality control (QC) tool in a whole exome sequencing workflow.

Mandatory for the experimental success of whole exome sequencing is the quality of the incoming genomic DNA (gDNA) material and the DNA samples at various stages of the library preparation workflow.

Methodology: Exome libraries were prepared according to the Agilent Low Input Sure-Select^{XT} Human All Exon v5 protocol from FFPE tumor tissue samples. To ensure success quality control was verified with an Agilent 4200 TapeStation system of the received gDNA samples and during the library preparation.

Results: Intermediate QC steps were taken throughout the protocol to monitor library preparation for sequencing, such as evaluation of DNA after fragmentation, analysis of adapter-ligated and amplified DNA, and lastly, qualification of the final library. The initial QC of incoming gDNA was determined based on the DNA integrity number (DIN). All samples had a low DNA integrity, what is usual for DNA extracted from FPPE material.

Conclusions: Quality control is an important part of NGS workflows, library preparation protocols recommend quantification and qualification of the DNA samples at various stages. The increasing sample throughput creates a need for automation especially in a core facility where many precious samples are proceeded with time pressure. The implementation of the automated electrophoresis system enabled to increase the efficiency of the workflow and ensure good sequencing results.

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P14.061A

Adopting hybridisation-based enrichment in molecular diagnostics of complex disorders via next-generation ion semiconductor sequencing

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Introduction: In current paradigm of next-generation sequencing, the ease of use and hands-on time in laboratory settings are the main factors in adopting IonTorrent semiconductor-based sequencing platform. The enrichment strategies for diagnostic panels are amplification-based (AmpliSeq), which creates a potential for pseudogene interference or compositional biases. An alternative choice is use of hybridisation-based approaches such as SureSelect enrichment protocol (Agilent).

Materials and Methods: Two separate panels were adopted, optimised and tested as a proof-of-concept: ClearSeq Comprehensive Cancer (4 samples) and a custom SureSelect panel designed for diagnostics of syndromic and non-syndromic forms of craniosynostoses (14 samples). The Next Generation Sequencing was performed using IonTorrent S5 platform.

Results: The following quality control parameters were achieved for both panels: coverage - 97%, on target – 74%, mean depth – 274, ISP loading – 80%, 97% aligned specifically. Bioinformatic analysis was performed on the Ion Reporter™ Software and variants were classified based on the results obtained from several predictors (e.g. PolyPhen-2, SIFT, Mutation Taster, CADD). Previously known mutations were detected across both panels, confirming their effective performance.

Conclusions: Hybridisation-based enrichment is recognized as a valid alternative for amplification-based panels in diagnosis of complex disorders. Further optimisation of cost-effectiveness ratio should increase the viability of the approach and allow for better parallelisation of sequencing multiple samples. Presented results clearly indicate that it is possible to adopt hybridisation-based approaches such as SureSelect enrichment protocol (Agilent) on the IonTorrent S5 platform, what significantly widens the options for semiconductor based sequencing of clinical samples.

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P14.062B

Optimization of a next generation sequencing innovative protocol for the accurate detection of punctual mutations and copy number variants in children with intellectual disability and obesity

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Introduction: Whole-exome sequencing has considerably benefited the molecular diagnosis of patients with suspected Mendelian disorders, but its poor ability to accurately detect copy number variations (CNVs) remains a major limitation for sensitive genetic screening. We developed a next-generation sequencing strategy (CoDE-seq) enabling the accurate detection of both CNVs and punctual mutations in one step, and assessed CoDE-seq for the molecular diagnosis of intellectual disability and obesity.

Methods: CoDE-seq is based on an augmented whole-exome sequencing protocol, with probes distributed uniformly throughout the genome in addition to exome. This new method was validated in 40 patients for whom chromosomal DNA microarray was available. Subsequently, CNVs and punctual mutations were assessed in 82 children or young adults with suspected monogenic obesity and/or intellectual disability, and their available parents.

Results: CoDE-seq not only detected all CNVs (n=97) identified by chromosomal DNA microarrays but also found 84 additional CNVs, due to a better resolution. When compared to CoDE-seq and chromosomal DNA microarrays, whole-exome sequencing failed to detect 37% and 14% of CNVs, respectively. In the 82 patients with suspected Mendelian obesity and/or intellectual disability, a likely molecular diagnosis was achieved in more than 30% of the patients. Half of the genetic diagnoses were explained by pathogenic CNVs while the other half by pathogenic punctual mutations.

Conclusions: CoDE-seq has proven cost-efficient for the accurate detection of CNVs and punctual mutations. It avoids the sequential genetic screening approaches currently used in clinical practice, and is highly effective for the molecular diagnosis of intellectual disability and obesity.

M. Derhourhi: None.

P14.063C
The study of mutational status of estrogen-dependent MCF-7 breast cancer cells

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The experiments were performed on in vitro cultured estrogen-dependent MCF-7 breast cancer cells and MCF-7 sublines resistant to antiestrogen tamoxifen and/or metformin - MCF-7/T and MCF-7/M. Next generation sequencing (NGS) using commercial panel for gene targeted enrichment - Gene Reader Actionable Insights Tumor Panel (GRTP - 101X)- was performed on the platform QCI Analyser version 1.1. For reliable evaluation of gene mutations 1.5% of tumor cells in the sample are enough. DNA libraries under study were normalized by concentration followed by pooling. The quality of the reads obtained after sequencing was higher than Q25 for more than 70% of studied DNA samples. The numbers of the reads for DNA samples analyzed after filtering were 1 490 465 for MCF7, 1 195 387 for MCF7/M and 1 376 487 for MCF7/T. The software automatically performed a filtering of the fragments obtained on quality, adapter trimming and mapping of the fragments obtained according to reference genome hg19. Gene variants with statistical significance ($p = 1.0$ E-4) were selected by manual software.

The NGS sequencing of PIK3CA, ALK, EGFR, EGFR-AS1, ERBB2, ESR1 genes has revealed identical genetic aberrations - mutations in the cell lines (Table 1). The presence of PIK3CA mutation (c.1633G>A) may be associated to tamoxifen and metformin resistance in both sublines and parental line.

Table 1. Concordance of mutational status in cell lines MCF7, MCF7/M, MCF7/T.

Gene	MCF7	MCF7/M	MCF7/T
PIK3CA	c.1633G>A **Allele fraction: 66% (of 17311 reads)**	c.1633G>A **Allele fraction: 67% (of 12976 reads)**	c.1633G>A **Allele fraction: 65% (of 11224 reads)**
ALK	c.2535T>C **Allele fraction: 100% (of 8773 reads)**	c.2535T>C **Allele fraction: 100% (of 2210 reads)**	c.2535T>C **Allele fraction: 100% (of 3463 reads)**
EGFR	c.474C>T **Allele fraction: 99% (of 1121 reads)**	c.474C>T **Allele fraction: 99% (of 2520 reads)**	c.474C>T **Allele fraction: 99% (of 2606 reads)**
EGFR, EGFR-AS1	c.2361G>A **Allele fraction: 99% (of 1365 reads)**	c.2361G>A **Allele fraction: 100% (of 1489 reads)**	c.2361G>A **Allele fraction: 99% (of 2174 reads)**
EGFR	c.2982C>T **Allele fraction: 51% (of 4994 reads)**	c.2982C>T **Allele fraction: 41% (of 2128 reads)**	c.2982C>T **Allele fraction: 47% (of 1581 reads)**
ERBB2	c.1963A>G **Allele fraction: 100% (of 3402 reads)**	c.1963A>G **Allele fraction: 100% (of 2800 reads)**	c.1963A>G **Allele fraction: 100% (of 2231 reads)**
ERBB2	c.3508C>G **Allele fraction: 99% (of 1188 reads)**	c.3508C>G **Allele fraction: 99% (of 1076 reads)**	c.3508C>G **Allele fraction: 99% (of 2903 reads)**
ESR1	c.30T>C **Allele fraction: 50% (of 941 reads)**	c.30T>C **Allele fraction: 53% (of 735 reads)**	c.30T>C **Allele fraction: 46% (of 625 reads)**
ESR1	c.1782G>A **Allele fraction: 53% (of 23574 reads)**	c.1782G>A **Allele fraction: 51% (of 8793 reads)**	c.1782G>A **Allele fraction: 53% (of 6958 reads)**

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P14.064D

Exclusion of unreliably mapped reads from the results of Ion AmpliSeq targeted NGS

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Introduction: The use of the NGS is fraught with errors: not all of the identified genetic variants are true and can be confirmed by alternative methods. Incorrectly mapped reads are among the causes of NGS analysis errors. We believe that using Ion AmpliSeq Targeted Sequencing Technology enables to eliminate unreliably mapped reads algorithmically utilizing additional information about the genomic coordinates of targeted regions and primers used for amplification. Thus alignments that do not correspond to the experiment design can be excluded from the downstream analysis.

Materials and Methods: We have analyzed NGS data of 30 patients (lymphocytes DNA has been used to prepare Ion AmpliSeq Comprehensive Cancer Panel libraries) and compared variant calling results based on the initial set of reads and on the revised set obtained by excluding unreliable reads.

Results: We identified several groups of genetic variants: (1) observed in both sets, 6072 variants; (2) detected exclusively in the original data, 127 (systematic, false positive); detected in the revised data only, 63 (false negative, previously undetectable).

Conclusions: We conclude, that the use of additional information about the designed start and end of the targeted regions allows to reduce the number of false-positive genetic variants detected due to misleading reads and to detect new ones that were not previously detected due to a seemingly low allele frequency. Thus we can increase the quality of interpretation of the NGS data, which is especially important for DNA diagnostics.

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P14.065A

Improvement of the molecular diagnostics in disease genes by detection of genomic deletions/insertions including pathogenic mosaic and promoter variants from NGS data

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NGS in a clinical diagnostic setting evolved routine testing for many genetic diseases. The identification of single nucleotide polymorphisms (SNVs) and small insertions/deletions (InDels) in protein coding regions is straight ahead. However, reliable detection of larger genomic deletions and insertions from NGS enrichment data remains sophisticated, especially for those presenting as mosaicism and/or are located in regulatory regions. As structural variations (SVs) may vary significantly in size, their detection from short reads and distinction from NGS errors is challenging. Somatic mosaicism also present in leukocyte DNA is underestimated. NGS methods have the capacity to identify pathogenic mosaic variants, providing insights into their spectrum and underlining their importance for clinical molecular diagnosis. For a cohort of 400 patients with connective tissue diseases (290 cases, 34 genes) and neurocutaneous syndromes (50 cases, *NF1/SPRED1* gene; 60 cases, *TSC1/2* gene) the coding and to some extend the promotor regions were enriched in a custom design and analyzed by NGS (Agilent Sure Select QXT, Illumina NextSeq500). For the detection of SVs emerging as copy number variations (CNVs) affecting at least one target the analysis combined the application of ExomeDepth, CoNVaDING and XHMM. The intersection of at least two tools is highly sensitive for heterozygous and homozygous CNVs whereas pooled results of all tools indicate SVs present as mosaics. Preliminary results identified SVs representing one heterozygous *COL3A1* deletion and one heterozygous *TGFB3* duplication. Moreover, three SVs suspected to imply mosaics comprise one *COL5A1* two exon duplication, one complete *FLNA* gene deletion and one *TSC2* promoter deletion.

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P14.066B

Rapid WES as a routine diagnostic test for children from the neonatal intensive care unit

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Whole exome sequencing (WES) has proven to be a successful approach for solving a wide range of genetic disorders. We have offered this test since 2014 on a large scale in a diagnostic setting for various indications. Some of the limiting steps in the diagnostic setting are the high costs and a turnaround time of approximately 4 months, resulting in non-optimal clinical management, especially for children in the neonatal intensive care unit (NICU). In the past year we have set up a workflow for rapid WES testing in children from the NICU in Sophia Children's hospital. Initially, we included 9 children with different indications to optimize the workflow. The samples consisted of 3 trios, 1 duo and 5 single patients. Although different analysis pipelines had to be used, in 4 out of 9 cases we found the explanatory pathogenic mutation. The turnaround time was relatively high (average 8 weeks), but workflow adjustments decreased this to 4 weeks maximum. In the past 3 months we performed trio WES on 7 children from the NICU, using one uniform workflow with a multiple congenital anomaly panel of 2764 genes. When requested, we expanded the analysis to full exome. One certain diagnosis was made and at least 3 full exome analyses provided promising candidate genes. All reports were completed within 3 weeks. In conclusion, fast WES analysis for NICU children helps to establish an early definitive diagnosis. This experience will help us to implement a comparable workflow for prenatal testing later this year.

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P14.067C

Fetal fraction following selective reduction in twin pregnancies

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Objectives: Loss of a fetus in a multiple pregnancy is recognized to be a complicating factor when using maternal plasma for aneuploidy screening. Cell-free DNA (cfDNA) from a non-viable embryo or fetus is released into the maternal bloodstream and may lead to an increased chance of a discordant NIPT result; however, the nature of this biological process is not well understood. The objective of this study is to observe changes in fetal cfDNA over time in pregnancies with one non-viable twin.

Methods: We present a cohort of four patients with euploid twin pregnancies in which fetal reduction was performed. Maternal blood was obtained prior to the procedure and at sequential time points. cfDNA was isolated from maternal plasma and polymorphic DANSR assays were used to determine the fetal fraction in each sample.

Results: Fetal fraction was observed to decrease between time points in all cases but no consistent pattern was observed. In contrast to the average increase in fetal fraction over time previously reported in singleton pregnancies, no patient had a higher fetal fraction at the end of the series than the start, likely reflecting the loss of contributory fetal fraction from the co-twin.

Conclusions: In this small cohort, the fetal fraction of cfDNA dropped in the weeks following fetal reduction. Because a similar pattern may occur in pregnancies complicated by a spontaneous fetal reduction, a larger study, in terms of number of pregnancies followed and measurements per pregnancy, may provide additional data to more comprehensively describe these patterns.

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P14.068D

Validation of gene causality for neurological disorders by WES/WGS analyses in a diagnostic setting

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Introduction: Many newly suggested disease-gene associations result from findings in single patients or families. Independent confirmation/validation as well as definition of the corresponding clinical and mutational spectra is necessary before these variants/genes can be considered as causative.

Patients and Methods: We have performed whole exome sequencing (WES) and whole genome sequencing (WGS) as diagnostic tool for 10,859 and 1,118, respectively, index cases. With a systematic analysis of the data as stored in our database (CentoMD®) we aimed to validate the role of newly identified genes as causative for a specific disorder.

Results: Based on application of an ad-hoc bioinformatics pipeline, Sanger confirmation and evaluation of clinical information, we have identified bi-allelic causative variants for: 1) *GTPBP2* related to a neurodevelopmental phenotype with severe, early onset motor and cognitive impairment, 2) *NUDT2* causing a hypotonia-neurodevelopmental disorder, 3) *NKX6-2* related to spastic ataxia with hypomyelinating leukodystrophy and 4) *ACER3* related to early onset, progressive leukodystrophy.

Conclusions: We confirmed the pathogenicity of bi-allelic *GTPBP2, NUDT2, NKX6-2* and *ACER3* variants and broaden the related phenotypic spectra. Therefore, these genes should be added to the growing list of causative genes for hereditary neurodevelopmental disorders to be considered during molecular diagnosis.

		# families		# patients		# disease causing variants		
gene	first association with monogenic disease	Previously published	Centogene data	Previously published	Centogene data	Previously published	Centogene data	Extension of phenotype
GTPBP2	2016	1	5	3	5	1	5	yes
NUDT2	2017	1	3	2	5	1	1	yes
NKX6-2	2016	7	8	15	12	7	4	yes
ACER3	2016	1	2	2	2	1	2	yes
Total	**2016-2017**	**10**	**18**	**22**	**24**	**10**	**12**	**for every gene**

A.M. Bertoli Avella: A. Employment (full or part-time); Modest; Centogene. **Z. Yüksel:** A. Employment (full or part-time); Modest; Centogene AG. **H. Yavuz:** A. Employment (full or part-time); Modest; Centogene AG. **C. Baldi:** A. Employment (full or part-time); Modest; Centogene AG. **A. Marais:** A. Employment (full or part-time); Modest; Centogene AG. **J.M. Garcia-Aznar:** A. Employment (full or part-time); Modest; Centogene AG. **N.M. Grüning:** A. Employment (full or part-time); Modest; Centogene AG. **L. Abbasi Moheb:** A. Employment (full or part-time); Modest; Centogene AG. **O. Paknia:** A. Employment (full or part-time); Modest; Centogene AG. **K.K. Kandaswamy:** A. Employment (full or part-time); Modest; Centogene AG. **M. Werber:** A. Employment (full or part-time); Modest; Centogene AG. **M. Weiss:** A. Employment (full or part-time); Modest; Centogene AG. **G.E. Oprea:** A. Employment (full or part-time); Modest; Centogene AG. **F. Al-Hakami:** None. **N. Alshaikh:** None. **S. Alameer:** None. **A. Shawli:** None. **M.J. Marafi:** None. **A.A. Elmonairy:** None. **F. Al-Mulla:** None. **W. Al-Twaijri:** None. **K. Al-Thihli:** None. **A.M. Al Shamsi:** None. **H.A. Abdelrahman:** None. **L. Al-Gazali:** None. **A. Shukla:** None. **K.M. Girisha:** None. **M. Garshasbi:** None. **Y. Housawi:** None.

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P14.070B

Human mitochondrial DNA sequencing by Oxford Nanopore MinION

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Mitochondrial cytopathies can be caused by mitochondrial DNA (mtDNA) anomalies such as point variants or large-scale single deletions. In addition to these causative anomalies, multiple deletions of mtDNA can arise due to pathogenic variants in nuclear genes involved in the maintenance of mtDNA. These 3 types of anomalies (point variants, single and multiple deletions) are investigated for diagnostic purpose thanks to amplification of mtDNA by 2 long-range PCR, and sequencing by NGS short reads technology. However, the use of short reads does not allow to easily and accurately identify the deletion breakpoints at the nucleotide level.

In order to determine if long read sequencing technologies overcome this limitation, we perform mtDNA sequencing of several controls and patients with single or multiple deletions on Oxford Nanopore MinION.

This technology allowed an accurate identification at the nucleotide level of the breakpoints of the deletions, even in the case of complex multiple deletions. This breakpoint determination is useful for family testing in the case of single deletion. Furthermore, studies could also take advantage of this method to decipher the mechanisms of occurrence and to track the accumulation of mtDNA multiple deletions in mitochondrial or degenerative diseases, but also during normal aging.

In addition, despite a higher per base error rate in comparison with other NGS technologies, the accuracy of Oxford Nanopore MinION sequencing is sufficient to correctly determine the mitochondrial haplogroup of each patient. Due to the portability of MinION, it raises the possibility of in-the-field sequencing experiments for population genetics studies.

A. Trimouille: None. **C. Mouden:** None. **C. Boury:** None. **A. Courrèges:** None. **P. Fergelot:** None. **M. Martin-Négrier:** None.

P14.071C

Detection of phage nucleic acid in human blood plasma in data from NIPT protocol

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Introduction: Whole genome NIPT designed to detect chromosomal or subchromosomal aberrations in the fetus. NIPT assays based on whole genome sequencing are actually analyzing total present cell free DNA isolated from human plasma. Obtained sequencing data contains reads mapped to reference human genome, but also a significant number of unmapped reads, some of them belonging to viruses. The main objective of this study was to identify nucleic acids of phages, the most abundant organisms in biosphere, in NIPT data and to try to build viral contigs from the unmapped reads.

Materials and Methods: We sequenced total DNA and transcribed RNA (cDNA) from plasma of both healthy individuals and individuals with bacterial infection. In addition, we analyzed samples from the surface of mucous membrane for both groups. In analysis, human reads were removed and all remaining fragments were taxonomically labeled using metagenomic classifiers and phage mappers. Presence and composition of phages in samples were visualized using Krona graphs.

Results: We detected the presence of phage sequences in a small number of samples in both groups. We hypothesize that phages can be used as indicators of certain pathophysiological conditions.

Conclusions: NIPT data offers a lot of additional information and various bioinformatic approaches can be used to obtain additional results. Our results demonstrate the detection of phage sequences in the data which are usually discarded in common NIPT analyses.

I. Gazdaricová: None. **D. Smoľak:** None. **A. Balá:** None. **J. Budi:** A. Employment (full or part-time); Significant; Geneton Ltd., Comenius University Science Park, Bratislava, Slovakia. **J. Gazdarica:** A. Employment

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P14.072D

Chromosome microarray proficiency testing and analysis of quality metric data trends through an external quality assessment program for Australasian laboratories

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Chromosome microarrays are an essential tool for investigation of copy number changes in children with congenital anomalies and intellectual deficit. Attempts to standardise microarray testing have focused on establishing technical and clinical quality criteria, however external quality assessment programs are still needed. We report on a microarray proficiency testing program for Australasian laboratories. Quality metrics evaluated included analytical accuracy, result interpretation, report completeness, and laboratory performance data: sample numbers, success and abnormality rate and reporting times. Between 2009 and 2014 nine samples were dispatched with variable results for analytical accuracy (30-100%), correct interpretation (32-96%), and report completeness (30-92%). Laboratory performance data (2007-2014) showed an overall mean success rate of 99.2% and abnormality rate of 23.6%. Reporting times decreased from >90 days to <30 days for normal results and from >102 days to <35 days for abnormal results. Data trends showed a positive correlation with improvement for all these quality metrics, however only 'report completeness' and reporting times reached statistical significance. Whether the overall improvement in laboratory performance was due to participation in this program, or from accumulated laboratory experience over time, is not clear. Either way, the outcome is likely to assist referring clinicians and improve patient care.

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P14.073A

Development and clinical utility ofINGEMM custom panels

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Introduction: There are many ways to tackle the genetic study in patients in NGS. Custom panels offer better base-pair coverage, running times, costs and dataset handling than other NGS applications such as whole genome sequencing and whole exome sequencing. We present our workflow and results in more than 10,000 patients studied with 25 different INGEMM NGS custom panels.

Patients and Methods: Sequencing libraries are prepared with SeqCap EZ system (RocheNimblegen) using NEXTflex- DNA-barcodes (Bioo Scientific) and sequenced on a NextSeq500 platform (Illumina). The researcher has selected a group of genes involved in their pathologies. Each panel has a particular size, and in our workflow we combine different panels using NEXTflex-DNA-barcodes in the same run. Bioinformatic analysis was carried out by the Clinical Bioinformatics Unit of INGEMM center.

Results and Discussion: The diagnostic yield can change between the different custom panels and pathologies, but in general we observe a high level of diagnostic yield. Our biggest custom panel is the Clinical one, it contains 1,588 genes involved in intellectual disability, autism spectrum disorders and other common genetic disorders, the diagnostic yield is 50%. "BAF-complex panel" includes 96 genes involved in BAF-complex (Coffin-Siris syndrome, Nicolaides-Baraitser syndrome) and other genetic disorders with overlapping features: 45%. "Cardio-panel", 325 genes related with cardiovascular diseases, 29%. "Hepato panel" with 125 genes involved in genetic liver diseases: 28% and "Oft-panel", genetic ophthalmological diseases and 298 genes, 60%. To optimize the analyses, panel testing should be performed by bioinformaticians, clinicians and laboratory staff in close collaboration.

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P14.074B

Ultra-low coverage sequencing is the most accurate method for library quantification prior to post-pooling exome capture

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Introduction: Exome sequencing is a powerful tool for inherited disease diagnostics. The price of exome sequencing has reduced dramatically, but library preparation and target enrichment are still expensive. Post-pooling target capture decreases enrichment price per sample, but in this case pre-pooling quantification becomes crucial for accurate estimation of required sequencing data. Equal distribution of reads per sample gives a possibility to sequence more samples in a single run. On the other hand, sample to sample variation can result in either dropout of under covered samples, or increases per sample data requirements to eliminate variations. UV absorption, intercalating dyes, capillary electrophoresis and quantitative PCR are widely used for library quantification. In this study we compared accuracy of these popular methods with ultra-low coverage whole-genome sequencing (ULC-WGS).

Materials and Methods: DNA was extracted using QIAmp DNA mini kit (Qiagen). Libraries were prepared using NEBNext Ultra DNA Kit (NEB). We quantified libraries by Bioanalyzer 2100 (Agilent Technologies), different qPCR approaches, Qubit 3.0 (Life Technologies), 300PE sequencing at nano flowcell on Miseq (Illumina) with followed bioinformatic quantification. Libraries were pooled together and enriched using Focused Exome kit (Agilent Technologies) and sequenced on Illumina HiSeq 2500.

Results: We found that ULC-WGS is the most accurate library quantification method. This method significantly decrease sample to sample variations thus being the most cost-effective. We also found that insert size significantly influences capture efficiency.

Conclusions: Our results show that ultra-low coverage sequencing overcomes qPCR and other popular library quantification methods in accuracy and cost-effectiveness.

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P14.075C

Reinterpretation and reclassification in diagnostic Next-Generation Sequencing: current daily practices and dilemmas of Dutch clinical genomics laboratory professionals

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Introduction: Information on genetic findings is in rapid flux: as new evidence becomes available, genetic results may turn out more/less pathogenic than initially communicated by the laboratory, which can have important consequences for patients. How laboratory professionals deal with this constantly changing information remains unclear; therefore, this topic merits attention.

This is the first qualitative research into how reinterpretation (reviewing new evidence on variants) and reclassification (changing variants' initial classifications, e.g. VUS to pathogenic) functions in everyday genomics laboratory

practice, and into laboratory professionals' perspectives on ideal (future) practice.

Methods: Fifteen laboratory specialists from all (nine) Dutch genomics laboratories participated in a 7-day online focus group. Brief follow-up telephone interviews were conducted with participants.

Results:

• No formal local/national reinterpretation policies or guidelines exist, despite participants' expressed.

• Participants identified a lack of phenotypic information updates from clinic to laboratory

• Practices among different laboratories appear similar:

1. Variants are reinterpreted ad hoc (commonly at request of clinicians or following identification of a previously detected VUS in a new proband) and never periodically.

2. Variants are reclassified depending on new evidence.

3.Clinicians are recontacted by laboratories when reclassifications have a potentially clinically relevant impact on patients' treatment/monitoring.

• However, important nuances are discernible: e.g. laboratory specialists differ in what is considered sufficient evidence for a reclassification, and what are deemed clinically relevant reclassifications that should be communicated to clinicians. This has important implications for guideline development, as it demonstrates that policy in this context may differ widely in its implementation.

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P14.076D

Multiplex, low-volume one-step RT-qPCR for gene expression analysis using the IntelliQube® and BHQ® probes

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One-step reverse transcription-PCR (RT-PCR) is widely used for gene expression analysis, RNA virus detection, and routine RNA quantification experiments.

In this study, we evaluated the IntelliQube® real-time PCR instrument for multiplex gene expression analysis using commercially available RNA from human liver, lung, and kidney tissue, a one-step RT-PCR master mix, and BHQ® probe-based assays from LGC Biosearch Technologies. BHQ probes and primers were designed to target the mucin 1 (MUC1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the TATA-box binding protein (TBP) in a triplex PCR reaction. The IntelliQube utilizes the Array Tape® consumable and combines liquid handling with real-time qPCR analysis in 1.6 μL reaction volumes.

Using TBP and GAPDH as reference genes, it was determined that Muc1 was upregulated in kidney 143-fold and lung 297-fold in comparison to liver, which correlated with previously published data. Using a three way ANOVA, it was shown that there was no significant difference in results between singleplex and triplex reaction formats, or between multiple runs on the IntelliQube.

This study demonstrates the ability of this instrument and the associated Array Tape technology to successfully multiplex BHQ qPCR assays in a one-step RT-PCR format for gene expression studies, without compromising data quality. Combined with the automated inline process and economic benefits of Array Tape, the IntelliQube and associated BHQ probe chemistry may prove to be a very useful platform for multiplex qPCR applications in Human Genetics.

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P14.077A

Copan buccal collection devices for Next Generation Sequencing analysis

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Background: Since Next-Generation Sequencing (NGS) and buccal samples are more and more used in Genetic field, Copan developed buccal collection and preservation devices for Genetic applications: human DNA (hDNA) free FLOQSwabs® (FS) available W/ or W/O Active Drying System; eNAT®, a molecular medium preserving nucleic acids at room temperature, and NAO® (nucleic acid optimizer) basket for complete sample recovery from the swab. The objective of this study was to demonstrate the performance of Copan collection and preservation devices for Genetic diagnosis and NGS analysis, including clinical exome (~6,300 genes).

Methods: Buccal swabs (BS), collected with dry FS and with FS stored in eNAT®, were extracted using QIAamp® DNA Mini Kit (Qiagen) and processed using the NAO® basket. Extracted hDNA was analyzed by Real-Time PCR, by electrophoresis on agarose gel and by NanoDrop Spectrophotometer (Thermo Fisher). DNA extracted from 3 dry FS and 3 in eNAT® BS, tested 4 weeks post collection, were used to analyze clinical exome using NGS technique.

Results: All the samples gave appropriate quantity and purity of hDNA (low 16, medium 40 and high 77 μg of hDNA/swab) and no DNA degradation on gel. High-throughput NGS performed on the extracted DNA generated results at quality levels as NGS results performed using EDTA-blood.

Conclusions: Data obtained in this study demonstrated that good quantity and quality of DNA can be recovered from buccal swabs collected with the Copan hDNA free FLOQSwabs® stored dry or in eNAT®medium. DNA recovered is also optimal for NGS analyses such, as clinical exome sequencing.

A. Squassina: A. Employment (full or part-time); Significant; COPAN ITALIA. **A. Gervasoni:** A. Employment (full or part-time); Significant; COPAN ITALIA. **M. Rosso:** A. Employment (full or part-time); Significant; COPAN ITALIA. **S. Castriciano:** A. Employment (full or part-time); Significant; COPAN ITALIA. **A. Breda Klobus:** A. Employment (full or part-time); Significant; Breda Genetics srl.

P14.078B

Accuracy of DNA Sequence and Quality Values for a new Sanger Sequencing Instrument

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Introduction: Sanger Sequencing with capillary electrophoresis is a cost-effective, time-efficient, and highly accurate DNA sequencing method to interrogate DNA for Mendelian variants and minor variants present at 5% or greater, using Minor Variant Finder software. With the introduction of the easy-to-use Applied Biosystems SeqStudio Genetic Analyzer, featuring updated KB Basecaller, new spectral auto-calibration algorithm, and integrated cartridge containing a new separation polymer, testing the accuracy of basecalls and Quality Values of SeqStudio sequence data is important.

Materials and Methods: Statistically sound validation of basecalling and QV accuracy requires generation of a significant number of diverse sequences. We used purified plasmids derived from shotgun sequencing of NA12878 NIST genome reference standard for de-novo sequencing as well as PCR resequencing of 38 amplicon targets, ranging in length from 143-873 bases, in up to 50 human specimens for mixed-base calling. 10,000 samples were generated, totaling 6,400,000 basecalls. Sequencing reads were tested for basecalling and quality value accuracy.

Results: The median error rate ranged from 0.25-0.48% in Q20-trimmed de-novo sequencing, depending on sequencing chemistry and short(350bp)-, medium(500bp)-, and long(800bp)-read sequencing options. The percent of correctly called mixed bases in resequencing data was greater than 99%; the false negative rate was 0.6%. Homopolymer stretches of length 31 were sequenced without error. The 5' end of amplicons sequenced with BigDye Direct chemistry were sequenced flawlessly starting at the first base.

Conclusions: Testing demonstrated robust performance for basecalling and QV accuracy on a wide variety of sequences generated on the new SeqStudio Genetic Analyzer.

Y. Chu: A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **S. Schneider:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **S. Berosik:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **J. Bodeau:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **S. Chang:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **D. Rodriguez:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **D. Woo:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **D.A. Hom:** None.

P14.080D

Expanding the spectrum of *SHOX* mutations in idiopathic short stature patients through a custom array-CGH

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The short stature homeobox-containing gene (*SHOX*) resides within the pseudoautosomal region 1 (PAR1) on the sex chromosomes, and *SHOX* defects are responsible for 2-15% of idiopathic short stature (ISS) cases. Molecular diagnosis for *SHOX* deficiency is carried out by sequencing the coding exons and by performing MLPA for deletions/duplications. We screened 700 ISS patients for *SHOX* defects for diagnostic purposes; fifty-four patients (7.7%) carried mutations. Among these, one patient showed a single probe deletion at homozygous state that did not involve any of the known enhancers. Since MLPA probes are enriched in the exons and in the known enhancers, we hypothesised that they might be other non-coding elements with potential regulatory activity not yet described. To test our hypothesis, we developed a high-resolution custom

array-CGH platform with 8000 probes within the PAR1 and we screened 50 individuals that tested negative with the standard methods. Two deletions of 12.3 kb (1 patient) and 7 kb (2 patients) downstream *SHOX* not present in 300 controls were identified. These rearrangements shared a 1.8 kb region, while the 12.3 kb deletion included a 1 kb region conserved among different species. A functional assay to test if these sequences affect the gene expression was performed in NIH3T3 cells. We observed an increase of luciferase activity (45%) for the construct harbouring the 1.8 kb region compared to the promoter vector ($p = 0{,}0003$). In conclusion, we identified a novel region downstream of *SHOX* with potential regulatory activity that might be responsible for ISS cases undiagnosed through standard procedures.

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P14.081A

Accurate calls for copy number variation using the Novallele™ HRM Analyzer

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Introduction: The Novallele HRM Analyzer is web-based software with comprehensive algorithms capable of analyzing and displaying high-resolution melting (HRM) data for copy number variation (CNV) assessment. We investigated two analysis methods using the software.

Methods: The first method requires multiple, characterized DNA controls from cell lines. Review of the difference plot using the software with the unknown samples overlaid on the DNA controls suffices to assign a copy number. The second method requires only one characterized 2-copy control prepared uniformly with the samples. A copy number is assigned to each sample using both the number of melt peaks on the derivative plot and sample fluorescence relative to the control on the difference plot. Samples were extracted using four different commercial kits. Both methods were investigated using SMN1 (n=1462) and SMN2 (n=1557) melt curves generated on five different types of thermocyclers.

Results: Both methods were 100% accurate. CNV calls were accurately assigned using all five thermocyclers and all four sample extraction kits.

Conclusions: The first method is simpler as compared to the second method and allows a user to immediately perform their experiments using commercially available controls. The advantage of the second method is that it uses a single, characterized control prepared with the samples, which avoids requiring multiple control wells. Both CNV analysis methods using the Novallele HRM Analyzer software generate accurate CNV calls. *This product is only available in the U.S.A. The products mentioned are for Research Use Only. Not for use in diagnostic procedures.*

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P14.082B

Quantitative fluorescent polymerase chain reaction (QF-PCR) for rapid and culture independent aneuploidy analysis in spontaneous miscarriages

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Spontaneous miscarriages occur in 10-40% of pregnancies. The high level of miscarriage can be related to difficulties in embryonic development caused by anomalies in the embryo itself. It is well known that a large proportion of first-trimester spontaneous abortions is caused by chromosomal disorders (40-64%): autosomal trisomies are most frequently detected, followed by monosomy X and polyploidies. The results of conventional cytogenetic studies of spontaneous abortions depend on tissue culturing and are associated with a significant cell culture failure rate related to necrotic tissues, low mitotic rate and maternal cell contamination. Cytogenetic studies show high rates of failure and chromosomal analysis is possible only in a low number of samples (20 - 30%). Extended QF-PCR analysis of chromosomes 13,15,16,18,21,22, X and Y was used for the rapid analysis of the most frequent chromosomal disorders in spontaneous miscarriage in order to overcome the problems connected with karyotyping such as culture failure, external contamination and selective growth of maternal cells. The results of QF-PCR have been compared with those of classical karyotyping. Since 2009 karyotyping in first-trimester miscarriage has been replaced by DNA analysis by QF-PCR. In about 98% of cases a report has been produced; in almost 50% of them a genetic aneuploidy has been identified as the genetic cause of miscarriage. In order to estimate the recurrence risk of those women in which the genetic cause of the miscarriage has not been detected by QF-PCR, we are taking into consideration a CGH Array analysis.

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P14.083C

Building high quality, chromosome-scale, *de novo* genome assemblies by Bionano genome mapping and NGS sequencing

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Diverse genetic variations exits among individuals and are crucial to the understanding of human biology and diseases. Numerous efforts are underway to generate high-quality reference genomes for different ethnic groups. However, human genomes are complex and contain large fractions of repetitive sequences that make generating high-quality assemblies difficult. Bionano genome mapping provides a solution to visualize genome structure. The synergistic approach of combining next-generation sequencing (NGS) data with Bionano maps produces affordable, contiguous, and accurate *de novo* genome assemblies.

Here, we present an improvement to Bionano's traditional nickase-based approach by employing an enzymatic direct labeling approach. This new method doesn't introduce any undesirable breaks in the DNA and allows us to create very contiguous Bionano maps which can then be used to scaffold NGS sequence assemblies to produce highly contiguous and structurally accurate hybrid assemblies that can span most repeat regions. This direct labeling is compatible with a vast array of various organisms.

We validated our approach with the well-studied human NA12878 genome. Starting with NGS assemblies with N50 ranging from 0.18 - 14.5 Mbp, we produced hybrid assemblies with N50 from 70 to 80 Mbp. Chromosome-arm length scaffolds were assembled in 20 out of 23 chromosomes, and alignments show that they were consistent with the hg19 reference. The hybrid assemblies incorporated 80-97% of total NGS sequences with over 99% scaffolding accuracy. The scaffolds generated with this data have set a new standard for genome assembly that can be accomplished in less than one week and starting at 500 dollars.

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P14.084D

Disease associated tandem repeat genotyping from NGS target sequencing data

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Repetitive DNA sequence represented a big technical challenge for NGS technologies. Their analysis istraditionally based on PCR-amplification followed by fluorescence capillary electrophoresis to identifyfragment length differences. Theoretically, NGS technology could offer several potential advantages forTandem Repeat Polymorphisms (TRPs) genotyping.Our aim was to test the feasibility of the analysis of TRPs in NGS data. To do this we compared 16 differentdisease-associated TRPs genotypes from short-read NGS data to those derived through fluorescencefragment analysis approach.We sequenced and analyzed 14 DNA samples affected by various neurodegenerative diseases with repeatexpansions of different lengths for 16 disease associated TRPs by Illumina MiSeq sequencing platform (300+300 bp reads). We performed a repeat specific capture probes design (Duitama 2014) which uses theflanking regions of the repeats to design enrichment probes in unique regions. For the target regionsenrichment, we used SureSelect XT Kit, (Agilent technologies). Besides a known bioinformatics tool(lobSTR), a new software procedure was successfully implemented to precisely genotype TRPs from NGSdata to achieve genotype accuracy and efficiency. Overall, we were able to compare 224 genotypes. Locus-specific TRP typing demonstrated a very good correlation (up to 80%) between genotypes derived byfluorescence fragment analysis and those measured by our NGS approach. Even for very large expansionswe have been able to identify the samples carrying a pathological expansion. This pilot experimentdemonstrated the feasibility of the analysis of TRPs in NGS data for a large number of loci.

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P14.085A

A Next generation analysis of mutation hot spots in CFTR, MTHFR and HFE

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Specific point mutations in HFE or MTHFR give rise to Haemochromatosis type 1 or Homocystinuria respectively, for both genes it is not feasible nor cost efficient to routinely screen the complete coding region. Although the CFTR mutation spectrum is more variable, a population bias is observed and targeted mutation analysis of preselected mutations is frequently offered as a first line test. Numerous commercially available kits were launched to analyse predefined mutations in these genes. In an era where the majority of routine diagnostic analyses moved to Next generation sequencing, we developed a (multiplex) NGS approach to include CFTR, HFE and MTHFR targeted mutation analysis in our general workflow. In this workflow we specifically target two HFE polymorphisms, MTHFR c.677 C>T and the 50 most frequent CFTR mutations detected in the Belgian population. Library preparation is done with a modified Nextera XT protocol and sequencing on a Miseq. An intensive pooling strategy decreased the sequencing cost, down to the limit were single nucleotide sequencing with NGS becomes cost effective. With in house developed scripts analyzing only the nucleotides of interest, we generate patient specific reports that can easily be interpreted. A thorough validation of this workflow was performed with hundreds of samples resulting in an overall sensitivity (>99%) similar to Sanger sequencing and a positive predictive value of >99%. Furthermore, the independency of commercially available kits allows us to easily change or add targets to our panels.

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P14.087C

The Italian Telethon Undiagnosed diseases Program

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The Italian Telethon Undiagnosed Diseases Program (TUDP) mission is bestowing a molecular diagnosis to rare undiagnosed patients collected through a countrywide network of pediatric hubs. Patients are prioritized according to well defined criteria, described using standardized and shared tools (i. e. Human Phenotype Ontology, HPO) and made "matchable" with other cases stemming from other Undiagnosed Diseases Programs all over the world, in order to favor the finding of "second cases" and to confirm ultra rare causal variants. We focus on pediatric patients with complex or syndromic diseases, who are subjected to in-depth exome sequencing. A tabular output provides annotations of genes and variants with multiple predictions of their potential impact and observed frequency in external and internal control datasets. Selected variants of uncertain pathogenic significance are tested through functional studies to elucidate the disease causality and the pathogenetic mechanism. For instance, depending on the supposed phenotypic effect, cellular model created by gene-editing or non-mammalian vertebrate models such as medaka fish are developed and characterized. Among the 200 patients we have gathered discussed, approximately two thirds have been prioritized and subjected to whole exome sequencing as "trios" (case(s) and parents). Overall, the success rate of diagnosing of TUDP is about 50%. Thanks to a new phased exome sequencing technology, we will now be also able to determine separately the maternal and paternal haplotypes in order to detect elusive mutations such as single exon deletions, polymorphic repeats and others.

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P14.088D

Initiative on Rare and Undiagnosed Diseases in Pediatrics (IRUD-P) in Japan: recent achievement and statistics

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The Initiative on Rare and Undiagnosed Diseases (IRUD) is a national consortium designed to help patients and their families suffering from rare and undiagnosed disease conditions in Japan. The project started in July 2015. The aims of the project are to make diagnosis on patients with rare and undiagnosed diseases, to construct their genome database with clinical information, and to make banking system of precious specimens. The pediatric version of IRUD (IRUD-P) is coordinated by dual centers, the National Center for Child Health and Development (NCCHD) and Keio University. The IRUD-P developed a nation-wide network for patient recruitment involving 17 regional core clinical centers, and mainly performed whole exome sequencing on children with undiagnosed diseases and their parents. Till now, more than 2,000 patients, who passed the first screening in the core clinical centers, were consulted to the IRUD-P centers. Specimens accompanied with medical information (n=6,600) were collected from patients and their families, mainly in trios, and sent to the centers. Of 882 patients analyzed, genetically confirmed diagnosis in 295 patients (diagnostic yield was 33.4%). Nine patients were found to have pathogenic variants in novel genes. Patient cohort represents children with a wide range of undiagnosed disorders (malformation syndromes 49.4%, neuromuscular disorders 29.9%, skeletal and connective tissue diseases 6.6%, renal diseases 1.6%, liver diseases 1.3%, others 11.1%). For the remaining undiagnosed patients, the IRUD-P consortium has started data-sharing network system (IRUD-Exchange) and disease model analysis. The IRUD-P also built international collaboration and data sharing on rare and undiagnosed diseases.

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P14.089A

Solve-RD. Solving the unsolved Rare Diseases

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Solve-RD is a H2020 funded flagship EU project that brings together 21 partners from 10 countries and which will be running from 2018 to 2022. The main ambitions are (i) to solve large numbers of RD, for which a molecular cause is not know yet, by sophisticated combined Omics approaches, and (ii) to improve diagnostics of RD patients through a "genetic knowledge web". Solve-RD will pursue a clear visionary and integrated "beyond the exome" approach. The entire Solve-RD project has been motivated, designed and put together by a core group of four European Reference Networks. Solve-RD will deliver 7 main implementation steps: (i) Collect Phenotypes, (ii) New phenotype patterns, (iii) Re-analyse exomes / genomes, (iv) Novel molecular strategies, (v) Functional analysis, (iv) Clinical utility and (vii) Towards therapy. For analysis Solve-RD will apply data driven and expert driven approaches. We anticipate to increase diagnostic yield from 19.000 unsolved exomes/genomes by about 3-5%. Cohort specific innovative -omis strategies will be pursued, also addressing cost-effective issues. Analysis of more than 800 patients with highly peculiar phenotypes will highly increase the chance to find novel disease genes and novel disease mechanisms. We anticipate to solve more than 2.000 cases. Finding further matching patients will be secured by newly developed matchmaking approaches and by screening using MIPs technology in the more than 20.000 unclassified patients. For the first time in Europe we will also implement a novel brokerage structure connecting clinicians, gene discoverer and basic researcher to quickly verify novel genes and disease mechanisms.

O. Riess: None. **O. Solve-RD Consortium:** None.

P14.090BAnalytical validation of MPS diabetes assay with NIST and 1000G project reference materials

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Introduction: Most MPS-based assays yield false-positive and false-negative results. According to the CLIA and CAP recommendations laboratories have to validate the performance of tests before reporting patient results. This analytic validation includes wet-lab procedures as well as data analysis. Moreover, laboratories should perform revalidation after any assay alteration. This complex process of proving that an assay works as expected and consistently achieves the expected result has not yet been thoroughly described.

Material and Methods: We have sequenced NIST RM-8398 and 17 GBR (1000G project) reference materials with 24-genes diabetes panel (104,3kbp). Alignment and variant calling have been performed using VariFind™ Software. For the reference and experimental data, we have applied developed algorithm that selects regions with sequencing quality above the given threshold. The algorithm calculates common, reference and experimental data-specific variants in these regions. All calculations have been made for the diploid genome, i.e. homozygous variants have been counted as two independent variants.

Results: For the diabetes panel we have identified 629 common variants, 9 false-negative and 4 false-positive variants. Sensitivity and specificity are 98.1% and 98.9% respectively (95% CI). We have found 15 discordant bases among 1 174 744 total analyzed bases in 18 reference samples. General accuracy of the assay is above 99.9%.

Conclusions: We have developed streamlined automated validation approach for targeted MPS-based assays that is suitable to perform fast assay revalidation on any alteration in the bioinformatic pipeline or laboratory protocol. Developed validation algorithm has been tested on different sequencing platforms and multiple targeted panels.

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P14.091C

Trusted Variant eXchange: a database for secure sharing of variant classifications between trusted partners

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Introduction: Guidelines aim to standardize the classification of genetic variants for rare diseases and cancer. However, adherence to guidelines can vary greatly within a single healthcare system or country, leading to discordance in variant classification. International classification databases such as ClinVar can support the variant interpretation process, but issues of data incompleteness and inconsistency remain. To address this, we have developed the Trusted Variant eXchange, or TVX.

Materials and Methods: Requirements for TVX were collected and collated through a series of workshops and feedback cycles with our clinical partners through BigMed, a research project funded by the Norwegian Research Council. The database was designed with the following in mind: scalability, for varying data volumes; and adaptability, for evolving technological and regulatory needs. The database was built using components and microservices, based around blob and table storage, queues and functions.

Results: TVX database facilitates sharing of evidence-based classification of interpreted variants between trusted clinical diagnostic partners, focusing on data quality and conflict reporting. After authentication, partners can submit and search for variants with their associated classifications through a secure API. Entering all new variant classifications builds up a collective, high-quality knowledge base with high transparency and traceability, with the ability to share further to international databases. Discordances in classification are flagged and resolution facilitated through communication with the relevant partners.

Conclusions: Harmonization of variant classification is a priority for multi-site healthcare systems where equal access to quality healthcare is a goal. TVX enables such harmonization while continuously curating and accumulating expertise.

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P14.092D

Clinical reassessment of post-laboratory variant call format<VCF>files

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Background: Next generation sequencing has been leading the genetic study of human diseases for the past ten years, generating a huge amount of sequence variant data, which are stored in variant call format files. To our knowledge, there has been little discussion about the utility of VCF files for reanalysis.

Results: In this study, twenty samples were clinically reassessed using variant interpretation software. We reported seven cases (n = 20) differently from the outside laboratory. This accounts for almost 35% of all cases and is mainly due to the ability to gather more information about the patient's phenotype. One whole genome sequence case changed from inconclusive to negative. In addition, we identified variants related to the patient's phenotype in six cases; two of them were whole genome sequence and four were whole exome sequence, all reported as negative before the reanalysis.

Conclusions: Comprehensive phenotyping of individuals is crucial step in identifying candidate phenotype-related variants. We outline the benefit obtained from access to the patient's medical records and communication with referring physicians.

Keywords: report, variants, classification, vcf, reassessment, genomic, genetic, counseling, NGS.

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P14.093A

Identification of the post-zygotic mosaic nonsense mutation in *WDR45*gene leading to beta-propeller protein-associated neurodegeneration and defining sex chromosomal mosaicism at whole exome sequencing

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Introduction: Beta-propeller protein-associated neurodegeneration (BPAN) is a very rare X-linked dominant disease. BPAN has been associated with *WDR45* gene and identified almost exclusively in females. The clinical presentation of BPAN is marked with a biphasic disease course. Herein, we set out to characterize the genetic defect underlying the complex pheonotype in describe a novel, *WDR45* mutation by using WES and also aim to define the mosaic/heterozygous ratios of WES on sex chromosomes.

Case: 34 years old male patient with a medical history of delayed psychomotor development and intellectual disability. After age of 24 years he developed moving difficulty, slowness and contraction in the legs. The neurological examination revealed dystonia-parkinsonism.

Results and Discussion: WES was performed to patient and mosaic c.873C>G; p.Y291*(NM_007075) mutation was found. The variant was confirmed in mosaic state only in the patients' blood but other family members did not carry the mutation. However, it has been observed that there are other mosaic/heterozygous variants on X chromosome in exome data.

To figure out the percentage of heterozygous SNVs, the ratio number of heterozygous SNVs to total number of SNVs is calculated for chromosome X. In order to identify heterozygosity rates were calculated for 101 female and 89 male individuals. WES heterozygosity rates of sex chromosomes can be used for quality control or identify anomalies of sex chromosomes.

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P14.094B

How many homozygous mutations in exome sequencing data are true homozygous?

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Introduction: Compound heterozigosity for a point mutation and a deletion is a well-known pathogenic mechanism for recessive conditions, but its overall incidence has never been investigated. The aim of this study was to assess retrospectively the frequency of this genetic alteration in a large exome dataset from unselected cases.

Materials and Methods: We reviewed the exome data of 642 patient-parents trios analysed for diagnostic purpose during the last three years. The findings on the techniques employed to confirm the genetic alterations are discussed.

Results: Among the 642 patient-parents trios exome analyses, 9 probands (1,4%) showed a pathogenic homozygous point mutation although only one of the two parents in each trio proved to be a carrier. This findings suggested there must be another genetic alteration underpinning the homozygous status of the probands. Indeed, 6 patients showed deletions spanning one exon up to a whole gene that were confirmed by MLPA, 2 patients carried large deletions and one showed uniparental isodisomy of chromosome 2, detected by CGH- and SNP-array respectively.

Conclusions: Our data show that a small but significant percentage of recessive conditions can be caused by compound heterozigosity for point mutations and genomic rearrangements. This pathogenic mechanism can be suspected in exome data but need confirmation by different techniques, that should be tailored on a case by case basis. A unifying wide method to detect all possible genomic alterations is currently not available.

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P14.095C

Measuring coverage and accuracy of whole exome sequencing in clinical context

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Purpose: To evaluate the coverage and accuracy of whole exome sequencing (WES) across vendors.

Methods: Blood samples from three trios underwent WES at three vendors. Relative performance of the three WES services was measured for breadth and depth of coverage in clinically implicated genes. The false negative rates were estimated using the segregation pattern within each trio.

Results: Mean depth of coverage for all genes was 189.0, 124.9 and 38.3 for the three vendor services. Fifty-five of the ACMG 56 genes, but only 56 of 63 pharmacogenes were 100% covered at 10x in at least one of the nine individuals for all three vendors; however, there was substantial inter-individual variability. For the two vendors with mean depth of coverage >120x, analytic positive predictive values (aPPV) exceeded 99.1% for SNVs and homozygous indels, and sensitivities were 98.9 - 99.9%; however, heterozygous indels showed lower accuracy and sensitivity. Among the trios, false-negative rates in the offspring were 0.07 - 0.62% at well-covered variants concordantly called in both parents.

Conclusions: The current standard of 120x coverage for clinical WES may be insufficient for consistent breadth of coverage across the exome, and additional measures of sensitivity may be useful. Ordering clinicians and researchers would benefit from vendors' reports that estimate sensitivity and aPPV, including depth of coverage across the exome and across pre-specified genes of interest.

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P14.096D
Improving diagnostic yield of exome-sequencing through prioritization of genes with predicted HPO assignments

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Interpretation of genetic variants identified through whole exome sequencing remains challenging, due to many variants with unknown clinical significance. In order to improve the diagnostic yield, we first developed a method that can predict per gene what the likely phenotypic consequences are when mutated, by integrating gene expression data on 31,995 public RNA-seq samples. We subsequently used this information, along with phenotype information per patient, to prioritize genes that, when mutated, likely cause each of these phenotypes.

We tested our method using a cohort of 20 solved cases, who on average harbor 312 variants with a high CADD score and a low minor allele frequency (of which many are classified as variants of unknown significance). We show that our method successfully captures the true causal variant in 85% of the cases within the top 10 of prioritized variants. This indicates that our ranking algorithm helps to reduces the number of variants that need manual interpretation. We expect our algorithm to be particularly helpful for unsolved cases, where the causal mutation might not be in known disease-causing genes, but rather in other genes that are co-regulated with the known disease-causing genes and so-far remain ignored in current analyses.

Our web-based gene function predictions and HPO based gene prioritization method is freely available at http://www.genenetwork.nl/. GeneNetwork.nl also provides lookup functionality for predicted GO-terms, Reactome pathways and KEGG pathways, based on the co-regulation network of 31,995 samples.

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P14.097A
Power efficiency of research reanalyze of negative clinical WES data to identify new genes in Intellectual disability or Congenital anomalies

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The diagnostic yield of clinical whole-exome sequencing (WES) in intellectual disability (ID) and/or congenital anomalies (CA) is now about 30%, which means that 70% of patients remain without a molecular etiology. To go beyond the stringent criteria of the ACMG recommendations in variant interpretation, which are limited to established human disease genes, a further analysis in a research environment appears essential to identify novel disease

genes. Thus, we performed a systematic research analysis of negative WES of 500 patients. The solo WES interpretation was extended through a translational research approach to all variants in order to identify candidate variants, by studying protein functions, model organisms, and a literature review. International data-sharing was used to identify additional patients carrying variants in the same gene, in order to draw definitive conclusions on their implication in the disease.

With this strategy, we identified or contributed for the identification of 45 disease-causing genes or candidates. The genes were classified in five categories: 1) new genes (17 genes); 2) new genotype-phenotype correlations for known genes supported by data-sharing (5 genes); 3) ultra-rare disorders with low recurrence (8 genes); 4) non-OMIM genes recently published (5 genes); 5) candidate genes (10 genes). This study demonstrates the power of research reanalysis after negative clinical WES and shows that screening results can rapidly lead to diagnosis. Despite using omics technologies, such as whole-genome sequencing, many new genes implicated in rare human diseases remain to be identified. The performance of WES will certainly improve in the future.

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High-throughput sequencing of Mendelian disorders: From raw data to diagnosis with lifetime value

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Introduction: High-throughput sequencing (HTS) is widely used for clinical applications such as the molecular diagnosis of Mendelian disorders. As the applied technology/workflow substantially affects the diagnostic yield, knowledge about the pitfalls and advantages of HTS technologies and analysis pipelines is crucial for the successful application of hitherto unprecedented large-scale genetic testing.

Materials and Methods: We address the chances and challenges of HTS in the molecular diagnosis of Mendelian disorders as well as assess the sensitivity/recall, precision, computation time, and disk footprint of four corresponding HTS analysis pipelines.

Results: We exemplify the limitations of targeted (gene panel) and whole-exome sequencing (WES) as well as emphasize the potential of whole-genome sequencing (WGS) in the detection of single nucleotide variants (SNVs) and copy number variations (CNVs). In addition, we elucidate limitations of short-read HTS on exemplary cases including the influence of homologous/repetitive regions (mappability <1) on variant calling and the impact of sequence composition on read depth, as well as show differences in the performance of WGS analysis pipelines.

Conclusions: We recommend to select the HTS method with care and to combine more than one independent bioinformatics pipeline for the most comprehensive data analysis. The use of PCR-free WGS (>60×) instead of WES or panels and the inclusion of CNV analysis can contribute to increased diagnostic yield in molecular diagnosis with lifetime value. As long-read HTS may overcome limitations of short-read HTS, it is envisioned as the future of (clinical) sequencing.

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Precise breakpoint detection of balanced and unbalanced structural variation in whole genome sequencing data using haplotype blocks created by linked-reads

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Introduction: Routine diagnostics of genome wide balanced and unbalanced structural variation is still dependent on classical techniques such as karyotyping and (SNP-)array. Both techniques result in a rough breakpoint detection where especially karyotyping can result in significant misinterpretation of chromosomal breakpoints. New sequencing technologies such as long range haplotyping by mapping linked-reads to generate haplotype blocks (Chromium Genome Solution, 10x Genomics, San Francisco USA) are expected to overcome this problem, although repeat sequence elements at breakpoints in the genome may occasionally interfere with a precise detection.

Materials and Methods: To test whether this NGS based approach with linked-reads is suitable for routine

diagnostics, two patients, one with a de novo balanced translocation (8;17) and one with a duplication detected with SNP-array, were analysed.

Results: The expected structural variants based on previous knowledge were identified and in both patients precise breakpoints of these variants could be detected. In the translocation patient, the breakpoint differed at least 15 Mb from the estimated breakpoint and proved *SOX9* to be causative for the phenotype of the patient. In the second patient the orientation of the duplication fragment could be defined to be in tandem, making a gene disruption to be causal for the phenotype unlikely.

Conclusions: Structural variant analysis using WGS with linked-reads promises to be a feasible technique for detection of balanced and unbalanced structural variants in the genome and may be a next step in replacing classical cytogenetic techniques for genome wide screening.

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P14.100D

Comparison of whole exome sequencing assays with boosted clinical content

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Utility of Whole Exome Sequencing (WES) in clinical diagnostics has been limited by the non-uniform sequencing coverage across exons, leaving a substantial proportion of the regions with shallow coverage that prevents accurate variant detection. We evaluated a WES assay that is specifically designed for clinical use, enables wide breadth of coverage resembling high-coverage gene-panel based assays, and provides high sensitivity in variant detection. We performed WES capture experiments using an assay with boosted clinical content, namely xGen Exome Research Panel (IDT) assay that was spiked-in with custom designed clinical content. Sequencing was performed using an Illumina NovaSeq sequencing system and data was down-sampled to 100M reads. Performance of the WES assay was demonstrated by using reference samples with high-quality variant calls (The Genome In a Bottle Consortium and Platinum Genome samples for SNVs and INDELs and Coriell samples for assessing Del/Dups and complex genetic variants). In clinically associated CCDS genes, the assay achieved high average sequencing depth (183x) and coverage (99.7% of regions covered >20x). Sensitivity to detect SNVs was 0.998, and for INDELs 0.97. Sensitivity to detect 1 exon deletions and duplications was 0.93 and 0.99 for 5 exon deletions and duplications. The assay was observed to provide a uniform coverage over difficult-to-sequence regions (e.g. the *RPGR* gene) and GC-rich 1st exons. Our results demonstrate that WES assay with boosted clinical content provide high sequencing coverage and allows high variant calling sensitivity for different genetic variations, which makes it well-suited for clinical diagnostics of inherited disorders.

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Assessment of in-solution enrichment capture protocols for whole-exome sequencing in HiSeq 4000 System

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Introduction: Whole-exome sequencing (WES) has become a central application in research and disease diagnosis. A performance comparison of the most common in solution-capture enrichment alternatives was needed in order to develop adapted cost-effective workflows for HiSeq 4000 System (Illumina).

Materials and Methods: Peripheral blood purified DNA samples were enriched with a reference kit (SureSelect-QXT, Agilent) and with various Illumina solutions: TruSeq-Nano, Nextera-DNA Exome with standard protocol (Nx-Std) and with various modifications (Nx-Mod), including insert sizes, input DNA and post-enrichment PCR cycles. Pools of 5-7 samples at 2 nM library concentrations were sequenced with 75 bp paired-end reads. Bcl2fastq (v2.18), BWA (v0.7.15), Samtools (v1.3), Picard (v2.10.10), QualiMap (v2.2.1), and GATK (v3.8) software were used for analysis.

Results and Conclusions: The best average enrichment (reads on target) was obtained for the Nx-Std solution (69%), although at distance from that provided by the reference kit (89%). However, Nx-Mod with 350 bp insert size had lower mean proportion of duplicate reads than the reference (23% *vs*. 25%) while returning a comparable proportion of target bases at >10X depth of coverage (84% *vs*. 91%). TruSeq-Nano performed the worst in all comparisons, except in the proportion of duplicate reads

(17%). At least for the proportion of duplicate reads, these results support that performance of HiSeq 4000 is not comparable to that of previous HiSeq systems, providing guidance for the design of WES projects.

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Implementing fast whole exome sequencing sequencing as diagnostic test for fetal multiple congenital anomalies on ultrasound

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Introduction: Identifying the cause of fetal anomalies seen on ultrasound provides important information to improve perinatal management. The conventional test (chromosomal microarray) leads to a diagnosis in approximately 25% of the fetuses with multiple congenital anomalies (MCA). Whole Exome Sequencing (WES) is a promising technique to improve diagnostic yield. Implementing WES in prenatal setting is challenging due to uncertainties around fetal phenotyping, variant interpretation, ethical issues of incidental findings and variants of unknown clinical significance and the requirement of short turnaround times.

Method:

Phase 1: Retrospective WES analysis (blindly) of six fetuses (including parents) with known postnatal genetic diagnosis to test if this diagnosis could be made on the fetal phenotype only. Variants were filtered using human phenotype ontology (HPO) or using our custom virtual gene panel (about 3850 disease genes, excluding late-onset diseases genes).

Phase 2: Prospective study of fast trio WES analysis in addition to conventional genetic tests for twenty-five fetuses with two or more congenital malformations.

Result:

Phase 1: WES analysis on fetal DNA resulted in a coverage > 95%. Five of six known diagnoses could be confirmed using our gene panel. HPO was not helpful. One causal pathogenic *PTPN11* mutation was missed due to low coverage of the variant. Modeling the WES pipeline shows a theoretical turn-around time of 10 days after the invasive procedure.

Phase 2: The prospective study has just started.

Conclusions: Retrospective analysis and modeling of our pipeline show that implementing WES as a routine test in the prenatal setting is technically feasible.

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Outcome of Whole Exome Sequencing for diagnostic cases of a clinic pediatric center: the Medical Genetic Unit of MeyerChildren's hospital experience

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WES has shown an unprecedented success rate in the identification of disease causing genes in projects ranging from tailored sequencing, used to discover the molecular bases of a recognizable syndrome in a homogeneous group of patients, to the systematic application of pan-genomic sequencing in large heterogeneous cohorts. The usefulness

of an unbiased sequencing approach has been highlighted in various heterogeneous disorders, including intellectual disability, developmental delay, kidney diseases and congenital heart defects. We analyzed 400 WES and 20 WGS on pediatric patients affected by different rare diseases. In particular, we studied patients with renal diseases, including pediatric kidney tumors, MODY, pediatric glioblastoma, Chiari Malformation-type 1, cardiomyopathies and patients with isolated/syndromic intellectual disability.In 54 trios with different clinical conditions (excluding renal diseases, MODY and Chiari malformation that showed greater diagnostic success) we obtained a diagnosis in more than a half cases, with a detection rate of 54%, while in a singleton analysis WES approach helped us to identify variants in causative genes on 15/21 cases. We showed that WES identified significantly more conclusive diagnoses than the standard care pathway without incurring higher costs helped also by the use of genotype-driven approach, as a complement to the traditional phenotype-driven one.Deep phenotyping and WES end a diagnostic odyssey, allows for precise genetic counselling and has the potential to change clinical management. It is also the start point for the development of targeted pharmacologic therapies, which can translate these discoveries into efficacious novel treatments to achieve a personalized genomic medicine.

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P14.105A

Does 10X Genomics technology improve the identification andcharacterization of structural variants?

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Structural variants (SV) include copy number variants (CNV) and balanced chromosomal abnormalities (BCA). Whole-genome sequencing (WGS) enables to detect SV at base-pair resolution. However, CNV and BCA are difficult to detect using a short-read sequencing, and long-read approaches are not yet available for diagnosis. Recently, 10XGenomics proposed a pseudo long-read technology, using linked-reads to reconstruct long DNA fragments. Since long read methods remain very expensive, 10X-Genomics could be a good alternative. The main aim of this work is to determine if 10X-Genomics solution enables a more sensitive detection and better comprehension of SV than a short read WGS. We included 13 patients with known or suspected SV, with signed informed consent: 5 possible chromoanagenesis, 7 reciprocal translocations and 1 roberstonian translocation. Whole-genome analyses were performed using Chromium (10X-Genomics) library preparation before HiSeq X (Illumina) sequencing, and compared to a classical HiSeq X sequencing. Two different pipelines were used, using BreakDancer for classical WGS and LongRanger for 10X-Genomics WGS. For BreakDancer, we used a local database filter out recurrent SV. The variant interpretations were blinded for bothtechnologies. The classical WGS pipeline allowed the diagnosis of known SV in 10/13 patients. The 10X-Genomics pipeline found 1/13 SV tagged as “high confident”, and 9/13 as “low confident”. None of them detected the roberstonian translocation. In conclusion, 10X-Genomics solution didn’t improve the SV detection in this small cohort. To increase the SV detection we point out the importance of local databases to filter recurrent SVs and the improvement of informatics pipelines using 10X-Genomics data.

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P14.106B

Utility of clinical Whole Genome Sequencing (WGS): diagnostic success factors now and into the future

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Introduction: Application of genomic technologies to genetically heterogeneous complex disorders significantly increases diagnostic yields. WGS further increases diagnostic success over other genomic technologies.

Methods: Genome.One is the world's first ISO15189 clinically-accredited WGS laboratory. In establishing this clinical laboratory we solved various challenges and identified potential directions to further leverage WGS for increasing clinical diagnoses.

Results: We reviewed clinical referrals from 400+ pedigrees (600+ individuals, 2/3 singleton referrals), with phenotypes ranging from intellectual disability to single organ disease. Many cases had extensive investigation (including microarray and exome sequencing) before referral. 52% had reportable findings and 32% met ACMG class 4 or 5. Incidental findings were reported in 3% of cases. Multidisciplinary review meetings enabled collaboration between geographically distant groups, enhancing variant curation and patient care.

Conclusions: We have largely solved issues relating to library preparation, sequencing and primary bioinformatics. Variant prioritisation and interpretation remain challenges we are addressing with filter automation and custom software solutions. The most promising diagnostic improvement (currently being implemented) is inclusion of structural/copy number variant detection with no lower size limits. Further improvements are likely with incremental inclusion of mitochondrial DNA analysis, automated data reinterpretation, and availability of secondary analysis (such as pharmacogenomics and Mendelian predisposition panels) to enhance the utility of genomic analysis.

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P14.107C

Low-coverage whole genome sequencing in plasma circulating cell-free DNA analysis: the Turner syndrome experience

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Turner syndrome (TS) is characterized by complete or partial absence of the second sex chromosome, either in mosaic or in all cells, in phenotypic female patients.

Gonadoblastoma risk is increased in TS with Y chromosome, thus gonadectomy is strongly recommended before starting growth-hormone treatment. The detection of Y chromosome, at low-level mosaic or as marker chromosome, may be tricky by standard karyotype (SK) and array-CGH (a-CGH). Thus, further molecular screening to detect Y-chromosomal sequences is mandatory in TS individuals who are negative by these approaches.

We performed low-coverage (0.2x) whole genome sequencing (lc-WGS) of plasma cell-free DNA (cf-DNA) to determine its potential role in TS diagnosis.

Our study was performed on 64 TS patients, previously characterized by SK and a-CGH analysis on genomic DNA, and 42 healthy controls (21 females, 21 males). Genome coverage information from controls was used as reference to identify structural variants.

We detected low-level mosaicism for XX or XY cell lines, partial deletions/duplications of sex chromosomes indicating an iso-chromosome, X chromosome partial deletions, some of them suggesting a ring/marker chromosome. WGS confirmed SK results in 50/64 cases; we detected nine X chromosome rearrangements at low-level mosaic that, although hidden at SK, were confirmed by a-CGH in all but 2 cases, and previously unreported Y chromosome material was found in 5 patients.

Our results show that, in clinical suspicion of TS, lc-WGS in cf-DNA is a valuable screening test for the detection of low-level mosaicism and complex structural chromosome abnormalities, in the face of extremely content cost.

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P15 Personalized/Predictive Medicine and Pharmacogenomics

P15.01A
Position of Croatian Roma in the global ADME core markers' variation landscape

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Introduction: The ADME (absorption, distribution, metabolism and excretion) genes' variation is markedly related to ethnicity and shows distinct geographic patterns. Generally, the knowledge on distribution of ADME genes in isolated populations is limited, particularly in the Roma, transnational minority population of Indian origin. The aim of this study is to determine the allele frequencies ADME "core list" markers and to compare them with world-wide data in order to elucidate the position of Roma in the global ADME genetic landscape.

Methods: The 95 loci from 32 ADME genes were genotyped using KASP method in 440 Croatian Roma DNA samples. Data were analyzed using standard statistical population-genetics methods.

Results: The analysis of genetic vs. geographic distances placed Croatian Roma among European populations but their proximity to South-Asian populations is also evident. Next, Roma show the outlying position on the global scale in minor allele frequencies of 12 loci: for 9 loci within 8 genes (rs1128503, rs1138272, rs1799853, rs1902023, rs3758581, rs8192709, rs10509681, rs12248560, rs34059508) they have the highest frequency while for three loci in three genes (rs28399433, rs28371725, rs4149117) have the lowest frequencies of the minor allele.

Conclusions: The outlying positon in almost 13% of ADME core markers results from the specific genetic history of the Roma population. This finding may be helpful in developing personalized medicines' protocols in drug therapies for this specific population. The research was funded by Croatian Science Foundation grant (HRZZ-IP-2014-09-4454) to MPS.

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P15.02B
Association of *ADORA3* gene polymorphisms with efficacy and toxicity of methotrexate

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Introduction: Adenosine A3 receptor (ADORA3) is part of the adenosine-mediated antiinflammatory pathway. These receptors are over-expressed in peripheral blood leukocytes and synovia of patients with rheumatoid arthritis (RA). Methotrexate (MTX), the anchor drug and part of the first treatment strategy in RA patients, exerts its antiinflamatory effects via increased release of adenosine into the

extracellular space. Adenosine binds to ADORA2a and A3 and initiates an antiinflammatory response. Therefore *ADORA3* gene polymorphisms could have an impact on MTX therapy outcome.

Materials and Methods: We have analyzed 118 RA patients on MTX monotherapy whose diagnoses were based on ACR/EULAR criteria. Genotypisation within *ADORA3* gene (rs3393, rs1544223, rs2298191) was performed using the KASP genotyping system. MTX efficacy was assessed based on the changes in the Disease activity score (DAS28) after 6 months of treatment according to the EULAR response criteria. Patients with good and moderate response were classified as ''responders'', whereas patients with poor response were considered ''nonresponders''. Data of adverse effects were collected during this period. MTX efficacy and toxicity were compared among patients with different genotypes.

Results: Among patients 106 (89,8%) were responders. Adverse effects were reported in 24 (20,3%) patients. There was no significant difference in therapy response between patients with different genotypes. Two patients had bone marrow complications, both were carriers of rare alleles of all three polymorphisms ($p = 0,02$).

Conclusions: According to our results, *ADORA3* gene polymorphisms may influence bone marrow toxicity in RA patients treated with MTX.

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P15.03C
Correction of splice mutation in COL6A1 gene with novel antisense oligonucleotides as prototype for other orphan geneticdiseases

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Premessenger RNA splicing is a necessary step in the production of a functional protein product. Many genetic variants lead to aberrant splicing and cause genetic diseases. One prominent technique for correcting splicing-related mutations is through the use of splice-switching antisense oligonucleotides (AONs). The antisense drug Nusinersen for SMA, recently approved by the FDA, is one notable example. Cummings et al. recently identified a highly recurrent pathogenic splice variant (c.904+189C>T) in *COL6A1* as a genetic cause of Collagen VI-related dystrophies (COL6-RD). The variant, located in intron 11, leads to the insertion of a dominantly-acting pseudoexon that disrupts the gene's critical motif. They estimate that ~25% of all cases with COL6-RD, but negative by exon sequencing are due to this mutation. This variant was found in a *de novo* pattern in over 30 patients, and we recently identified it in our lab in a 4 year old girl with COL6-RD. As part of *in silico* machine learning tools we are developing in our lab, we designed 16 AONs with 2'-OMe modifications and a phosphorothioate backbone to correct the c.904+189C>T variant. The most effective AONs were able to reduce the mutant allele by 95% in a dose dependent manner. The AONs designed in this work, as well as those designed in the independent work by Bolduc et al., may offer a treatment for children suffering from the collagen VI-like dystrophy. We expect the knowledge gained in this project to be applicable to a wide range of splice mutations. Grant support: Thrasher Research Fund

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P15.04D
Reporting genetic risks to participants of the Estonian Biobank, Genome Center

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Introduction: The Estonian Genome Center at the Institute of Genomics, University of Tartu, Estonia (EGCUT) holds genotype and health data of nearly ~52,000 individuals. At the end of 2017, EGCUT has started offering personalised genetic risk predictions to its participants. The reports include genetic risk scores for common diseases, carrier status, other hereditary risk factors, and pharmacogenetic recommendations.

Methods: To date, 47,000 biobank participants have been genotyped with the Illumina Global Screening Array and nearly 5,000 individuals have whole genome or exome

sequencing data available. Digital semi-automated report assembly and participant portal have been set up for signing an informed consent for return of results, scheduling the counselling appointment and updating one's records. For common complex diseases genetic risk scores (GRS) are calculated. For type 2 diabetes, 10-year risk estimates incorporating both GRS and classical risk factors are provided. Clinically actionable genetic variants are reported based on ACMG guidelines. The immediate and long-term feedback of the participants is surveyed to understand the impact of the return of results.

Results: 1283 participants have expressed interest through logging into the patient portal, 499 have registered for feedback visit and 209 have attended to the genetic counselling session from November 2017 to mid-February 2018, with an average of 24 participants in per week.

Conclusions: The EGCUT initiative of return of results to population biobank participants provides insight on how healthy individuals respond to personalised genetic risk information. The long-term aim of the project is to promote the introduction of personalised medicine in Estonia.

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P15.05A
Comprehensive prediction of responses to chemotherapies by biochemically-inspired machine learning

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Chemotherapy response varies significantly among cancer patients, and drug resistance is responsible for significant amount of this mortality. The patterns of gene expression and copy number changes in the tumour can predict treatment outcomes after chemotherapy by supervised machine learning (ML)[1-3]. Computational models based on transcriptome gene signatures of tumor cell lines were used predict chemosensitivity and resistance for 25 cancer drugs. This learned set of genes was used to predict clinical outcomes from tumor transcriptomes. Gene signatures have been derived for 5-fluorouracil, bortezomib, carboplatin, cisplatin, docetaxel, doxorubicin, erlotinib, epirubicin, etoposide, gefitinib, gemcitabine, hydroxycyclophosphamide, imatinib, irinotecan, Ixabepilone, methotrexate, oxaliplatin, paclitaxel, pemetrexed, rapamycin, sorafenib, tamoxifen, topotecan, vinblastine, and vinorelbine. These are comprised of 3 - 17 biochemically-relevant genes, and have misclassification rates from 0% to 26.1%. Validation is performed in patients with breast, bladder, ovarian, and colon cancers. Signatures derived from tumour cell lines predicted complete remission from paclitaxel treatment in 84% of breast cancer patients (10% more accurate than differential expression analysis). Expression of MAPT correlated with survival in paclitaxel-treated breast cancer patients. Cisplatin and hydroxycyclophosphamide-resistance were respectively predicted with 71% and 66% accuracy in bladder and breast cancer patients. Analysis of a comprehensive set ML-based gene signatures for a panel of drugs in primary tumours would be feasible to carry out prior to treatment, and could influence selection of therapy. If current treatment plans are not adequate, ML-based genomic profiling may also offer alternative tailored strategies for adjuvant chemotherapies. [1]Mol.Oncol. 10:85-100, 2016; [2]F1000Res. 5:2124, 2017; [3]bioRxiv. https://doi.org/10.1101/231712. Funding:NSERCDiscovery RGPIN-2015-06290.

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P15.06B
Novel copy-number variations in pharmacogenes contribute to interindividual differences in drug pharmacokinetics

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Introduction: Variability in pharmacokinetics and drug response is shaped by single-nucleotide variants (SNVs) as well as copy-number variants (CNVs) in genes with importance for drug absorption, distribution, metabolism, and excretion (ADME). While SNVs have been extensively studied, a systematic assessment of the CNV landscape in ADME genes is lacking.

Methods: We integrated data from 2,504 whole genomes from the 1000 Genomes Project and 59,898 exomes from the Exome Aggregation Consortium to identify CNVs in 208 relevant pharmacogenes.

Results: We describe novel exonic deletions and duplications in 201 (97%) of the pharmacogenes analyzed. The deletions are population-specific and frequencies range

from singletons up to 1%, accounting for >5% of all loss-of-function alleles in up to 42% of the genes studied. We experimentally confirmed novel deletions in CYP2C19, CYP4F2, and SLCO1B3 by Sanger sequencing and validated their allelic frequencies in selected populations.

Conclusions: CNVs are an additional source of pharmacogenetic variability with important implications for drug response and personalized therapy. This, together with the important contribution of rare alleles to the variability of pharmacogenes, emphasizes the necessity of comprehensive next-generation sequencing–based genotype identification for an accurate prediction of the genetic variability of drug pharmacokinetics.

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P15.07C

Innovative, high-sensitive and short-term Digital Droplet PCR (ddPCR) methodology development, for oncologic therapy response associated biomarkers quantification

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Introduction: Outstanding advances in pharmacogenetics knowledge and its influence in oncological therapy success has impelled the need of a high-sensitive and short turnaround-time methodologies for quantification of optimal drug treatment biomarkers. ddPCR technique has demonstrated the best sensitivity and turnaround-time/cost ratio. The aim of this work was to develop a ddPCR assay for biomarker quantification in *NRAS, KRAS, EGFR* and *BRAF* genes.

Materials and Methods: Mutant control fragments for twenty-nine selected high frequency-biomarkers were generated through directed mutagenesis and confirmed by Sanger Sequencing. The methodology was developed using allele frequency positive controls mixes for QX200 (BioRad) and QuantStudio™3D (Applied Biosystems) platforms. Quantification and detection limits and false positive rates were defined. To establish genotypic concordance designs were tested in Horizon lines and/or FFPE samples previously quantified.

Results: The design detected the complete biomarker-set. False positive rate, and detection and quantification means are described in the table. Selected variants were identified in both Horizon lines and clinical samples within a 0.4-1.6% quantification concordance.

Description of the obtained technical parameters

ddPCR	Sensitivity	Mean False positive Rate	Mean Limit of Detection	Mean Limit of Quantification	Turnaround Time
KRAS	0.001%	0.045%	0.657%	0.813%	1 day
NRAS		0.810%	1.190%	1.190%	
EGFR indels		0.084%	0.359%	0.459%	
EGFR SNVs		0.063%	0.187%	0.285%	
BRAF		0.279%	0.513%	0.513%	
Pyrosequencing (data described in published literature)	5%	1-5%	5-10%	5%	2 days
Sanger Sequencing (data described in published literature)	15%	5%	5%	5-10%	2-3 days

Conclusions: Our results show ddPCR as an ultrasensitive low time-processing technology with affordable requirements (simple designs, manageable procedures, suitability for complex samples). These features make ddPCR the optimal technique for biomarker quantification in oncological therapy, providing useful genetic information for drug selection. This testing translation to clinical routine would increase therapy effectiveness, shorten elapsed-time until treatment administering, and prevent adverse effects development.

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P15.08D

Pipeline for knowledge curation and decision support in pharmacogenomics

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The introduction of whole-genome sequencing in clinical practice has opened a path for preemptive, opportunistic pharmacogenomics (PGx) testing. The Pharmacogenomics

Knowledgebase (PharmGKB) distributes clinical dosing guidelines, and provides a link between these guidelines and genomic variants. Before applying this knowledge in healthcare, it is necessary to verify and adapt the guidelines to local practices. We describe a pipeline for translating knowledge from knowledge databases such as PharmGKB into a system that is valid for PGx decision support in our clinic.

We implement the pipeline using the Web Ontology Language (OWL) in order to keep the semantics of the decision support system compatible with PharmGKB knowledge. OWL ontologies allow for automatic testing of ontology coherence, and the inference of dosing recommendations based on the genomic variants in each patient.

We demonstrate how we have translated the semantics of the PharmGKB linked data model into an OWL ontology. We emphasize the strategy for aligning the PharmGKB definitions of indel and structural variants with patient variants. Then we outline how we use a web-based tool for collaborative curation of PGx knowledge. Finally, we show how we can provide curated dosing recommendations to whole-genome sequenced patients, exemplified by the drugs azathioprine and clopidogrel.

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P15.09A

Genetic profiling of voltage-gated sodium cannels in painful and painless diabetic neuropathy

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Diabetic patients can develop painful (PDN) or painless (PLDN) neuropathy irrespective of the severity or duration of diabetes. Understanding the underlying genetic background would allow developing personalized therapies. We examined the incidence of variants in ten Voltage-Gated Sodium Channels (VGSCs) genes in PDN/PLDN patients and analyzed if differences in frequency, protein localization or pathogenicity correlated with the phenotype stratification. VGSCs genes were sequenced by Molecular Inversion Probe-NGS and run on NextSeq500 Illumina©. The variant selection and the pathogenicity classification were performed according to the ACGS guidelines. We analyzed 547 patients: PDN (41.5%) and PLDN (58.5%). We identified 33 rare variants exclusively carried by PDN (N=38) and 42 exclusively carried by PLDN (N=47). The distribution of the mutations among the VGSCs genes is reported in the table. 6 PDN patients with more than 1 mutation were prevalently carrying *SCN9A* mutations whereas the 8 plurimutated PLDN patients were prevalently carrying *SCN10A* mutations. According to pathogenicity classification, 84.9% was represented by Class 3 mutations and 12.1% by Class 4 in the PDN cohort, whereas in the PLDN were 83.3% and 9.5% respectively. Considering the protein localization, PDN mostly carried variants located in the linker DII-DIII (83.3%), PLDN in the C-terminal (87.5%). This study describes the genetic profiling in PDN/PLDN neuropathy suggesting a preliminary approach for the phenotype stratification based on VGSCs genetic characterization.

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SYMBOL	PDN	PLDN	Total
SCN3A	2 (50 %)	2 (50 %)	4
SCN7A	7 (50 %)	7 (50 %)	14
SCN8A	1 (33.3 %)	2 (66.7 %)	3
SCN9A	4 (50 %)	4 (50 %)	8
SCN10A	7 (33.3%)	14 (66.7%)	21
SCN11A	5 (41.7 %)	7 (58.3 %)	12
SCN1B	2 (50 %)	2 (50 %)	4
SCN2B	2 (40 %)	3 (60 %)	5
SCN3B	2 (66.7 %)	1 (33.3 %)	3
SCN4B	1 (100 %)	0 (0 %)	1
Total	33 (44 %)	42 (56 %)	75

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P15.10B
Detection of Familial Chylomicronemia Syndrome in a cohort of patients with severe hypertriglyceridemia through a Next Generation Sequencing approach

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Aims: Familial Chylomicronemia syndrome (FCS) is a rare recessive disease caused by mutations in *LPL, APOC2, APOA5, LMF1* and *GPIHBP1* genes. It is characterized by very severe hypertriglyceridemia (HTG) with or without episodes of abdominal pain and recurrent acute pancreatitis. FCS diagnosis is often difficult due to its phenotypic similarity with other forms of severe hypertriglyceridemia. The aim of our study was to detect pathogenic mutations in candidate genes in patients with suspected FCS based on specific clinical criteria and evaluate clinical differences between genotypes.

Methods: By examining 3000 clinical records, 31 patients were classified as suspected FCS on the following criteria: *a)* plasma trigliceridemia (TG) levels >1000 mg/dl in multiple determinations; *b)* resistance to pharmacological therapy; *c)* history of acute pancreatitis. All patients underwent fully clinical examination and their information were collected retrospectively. Candidate genes were sequenced using Next generation sequencing (NGS) technique.

Results: 51.6% subjects were carriers of FCS causing mutations, the majority in *LPL* gene (56.2%). Compared to non-carriers, FCS patients showed higher prevalence of history of acute pancreatitis (P=0.04) and early onset of HTG (P=0.0008). Comparing homozygous carriers of mutations in other genes with *LPL* homozygotes, the latter group showed higher TG levels ($P < 0.001$) and lower TG reduction during treatment (P_{adj}=0.03).

Conclusions: Our data suggests that the proposed diagnostic criteria are highly predictive of FCS diagnosis in severe HTG patients. Mutations in *LPL* gene are the most common cause of FCS and homozygous carriers of mutations in *LPL* gene have the more severe clinical phenotype.

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P15.11C
Modulation of cGMP and cAMP as a new therapeutic target for Fragile X Syndrome

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Fragile X syndrome (FXS), the most common form of inherited intellectual disability and a leading cause of autism spectrum disorder (ASD). It is due to the functional deficiency of the Fragile X Mental Retardation Protein (FMRP), an RNA-binding protein involved in translational regulation of many proteins having key roles in synaptic morphology and plasticity. No specific and effective treatment for FXS is available. We searched for FMRP targets by HITS-CLIP during early development of multiple mouse brain regions (hippocampus, cortex and cerebellum) at a time when FMRP is most highly expressed and synaptogenesis peaks. Our data point out one specific phosphodiesterase mRNA as a prominent target of FMRP which negatively modulates its translation and intracellular transport. Since the abundance of this protein and activity are increased in *Fmr1*-KO cortex and hippocampus impacting the homeostasis of cAMP/cGMP, we propose here a new therapeutic approach for FXS, based on the specific pharmacological inhibition of this protein. We will present data showing pharmacological inhibition of this enzyme rescues some behavioral deficits in newborn and adolescent *Fmr1*-null mice such as social communication, discrimination and interaction. Importantly, chronic blockade in newborn *Fmr1*-KO mice, followed by a wash-out interval, results in the rescue of the altered social behavior in adolescent mice, showing that the beneficial effects of early pharmacological blockade of our target are long-lasting.

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P15.12D
Preliminary genomic analysis of the *GenoFit* cohort: a health and fitness study

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In addition to the elucidation of complex disease risk, genome wide association studies (GWAS) have potential to uncover the genetic components of health, fitness and wellness. Genetic variants associated with quantitative traits such as height, adiposity (BMI/waist hip ratio/visceral fat), blood pressure and hand grip strength have been uncovered in multiple studies. *GenoFit* is a recently initiated study of the genetic basis of health and fitness associated variation, collected among attendees of a university fitness centre. *GenoFit* participants are physically assessed for general health and fitness measures including bone and muscle health. Participants also complete a detailed lifestyle questionnaire. The age profile of the participants varies from the student population to locally resident individuals in their 70s and 80s. Fitness and adiposity metrics are approximately representative of the general population. The final target population is 5,000 individuals. The genomes of all enrolled individuals will be whole genome sequenced to a depth of 30x and genotyped using an Affymetrix precision medicine research array containing over 800,000 markers. To date, 277 individuals have been genotyped, and analysed using linear regression for quantitative trait association using PLINK v1.9. Analyses have yielded nominally significant (uncorrected $P < 0.05$) association signals for established genetic loci for adiposity (BMI/waist hip ratio: *FTO*, *MC4R*, *GNPDAP2*, *GRB14*, *CPEB4*, *FAM13A*). With the current sample size, no loci reached genome-wide significance. This non-obese Irish population sample is currently under active ongoing sample collection, phenotypic characterisation and molecular analysis with the aim of developing a valuable resource for future genomic studies.

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P15.13A

rs2648841 in miR-1208 is involved in hepatotoxicity during consolidation in childhood acute lymphoblastic leukemia treatment

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Background & Aims: Hepatotoxicity is one of the most common drug-related toxicity during the treatment of childhood acute lymphoblastic leukemia (ALL). During induction, this toxicity may be due to asparaginase while in consolidation is typically associated with methotrexate (MTX). Pharmacogenetic studies have identified SNPs strongly associated with this toxicity in children with ALL during the induction while during consolidation, most of the studies have analyzed few variants in MTX pathway genes, with no clear markers. Recently, we found association between another MTX-related toxicity, mucositis, and a SNP in miR-1206. Many genes involved in liver-specific signaling pathways are tightly controlled by miRNAs. In consequence, we hypothesized that variants in miRNAs targeting ASP-related genes in induction and MTX-related genes in consolidation could be also associated with drug-induced hepatotoxicity.

Methods: Therefore, we analyzed all the SNPs in miRNAs that could target these drug-related genes in a large cohort of Spanish children with ALL homogeneously treated.

Results: A total of 5 SNPs in 5 miRNAs during induction were associated with hepatotoxicity and 11 different SNPs in 10 miRNAs during consolidation, which suggests different mechanisms. Among them, we pointed out rs2648841 in miR-1208 which was the most significant SNP associated with hepatotoxicity during consolidation phase after Bonferroni correction, probably through a higher expression of its target genes in the MTX pharmacodynamic pathway, *DHFR*, *MTR* and *MTHFR*.

Conclusions: These results support the importance of miRNAs studies to understand the differences in toxicity to chemotherapy in children diagnosed with ALL.

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P15.15C

Generation of patient-specific hiPSCs model to study *POLD1*-related MDPL syndrome

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Mandibular hypoplasia, Deafness and Progeroid features with concomitant Lipodystrophy and insulin resistance, define a multisystem disorder named MDPL syndrome. MDPL has been associated to *de novo* heterozygous mutations in *POLD1* gene which encodes the active site of DNA polymerase δ, involved in DNA replication and repair mechanisms. Recent cellular studies revealed complex aetiology of this disorder, with an important role for POLD1 function in adipose homeostasis. To date, 22 cases with *POLD1*-related MDPL syndrome have been reported worldwide. In order to improve our understanding on the molecular mechanisms of the MDPL disease, we generated human induced pluripotent stem cells (hiPSCs) from dermal fibroblasts obtained from a patient harbouring the recurrent heterozygous in-frame deletion p.Ser605del within polymerase domain. The cells displayed expression of stemness markers by immunofluorescence studies and normal pluripotent stem cell morphology. In addition, despite MDPL- hiPSCs showed normal phenotypes without significant nuclear abnormalities, we highlighted the presence of micronuclei, that correlates with DNA damage repair defects and genome instability. Their subsequent differentiation into adipose tissue, that is mainly involved in the MDPL disease, could represent an outstanding opportunity to yield additional insights into the pathophysiology of fat loss. Finally, this new tool might be used for drug screening and further development of targeted therapeutic approaches.

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P15.17A

Confirming the identity of human cell lines and other ex vivo-manipulated human samples

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The study of human development and diseases relies on analysis of samples obtained and manipulated *ex vivo*. It is critical in such studies to know the provenance and confirm that the identity of these materials is correct. Analysis of highly variable short tandem repeats (STRs), provides a simple, inexpensive and highly specific genetic "fingerprint" of a human sample. In this study, we describe a complete workflow for cell line authentication and chimeric receptor T-cell (CAR T) and induced pluripotent stem cell (iPSC) sample matching. Analysis of serial dilutions of purified genomic DNA from isolated human cell lines identified the correct alleles from as little as 0.1ng of purified DNA. Samples immobilized onto Copan NUCLEIC Cards were correctly identified when a suspension of 5-10 x 10^5 cells were dried onto the cards. Contaminating HeLa alleles could be detected in a mixture when they were present in as little as 1% of genomic DNA from this population. Blinded samples of donor and iPSCs, as well as manipulated CAR T cells, were correctly matched in every case tested. Together, these results describe a facile and consolidated workflow for establishing the provenance, authenticity and identity of *ex vivo*-obtained human samples.

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P15.18B

Immune checkpoints PD-1 and PD-L1 polymorphisms and outcome of cisplatin-based chemotherapy in malignant mesothelioma

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Introduction: Immune checkpoints such as programmed cell death 1 (PD-1) or programmed cell death 1 ligand 1 (PD-L1) are key modulators of a balanced physiological immune response. Cancer cells can exploit these mechanisms to evade immune response and PD-1 or PD-L1 inhibitors have recently proved successful in several tumors. However, PD-1 and PD-L1 expression may also confer resistance to cisplatin-based chemotherapy, commonly used in treatment of malignant mesothelioma (MM). Our aim was therefore to determine whether polymorphisms in immune checkpoint genes influence treatment outcome in MM patients.

Materials and Methods: MM patients treated with gemcitabine/cisplatin or pemetrexed/cisplatin doublet chemotherapy were genotyped for six polymorphisms in genes coding for PD-1 (*PDCD1*) and PD-L1 (*CD274*). Cox and logistic regression were used to assess their influence on treatment outcome.

Results: Among 171 MM patients, carriers of at least one polymorphic *CD274* rs4742098 (c.*2635A>G) allele were more likely to achieve partial or complete response after gemcitabine/cisplatin treatment compared to carriers of two wild-type alleles (OR=2.19, 95% CI=1.02-4.71, P=0.045). They also had significantly longer progression-free (9.1 vs 7.1 months, HR=0.64, 95% CI=0.43-0.94, P=0.025) and overall survival (21.5 vs 14.0 months, HR=0.59, 95% CI=0.39-0.91, P=0.016). Regarding adverse events, *CD274* rs4742098 was associated with increased risk of nausea/vomiting (P=0.024) and alopecia (P=0.036). *CD274* rs4143815 and *PDCD1* rs10204525 were also associated with increased risk of nausea/vomiting (P=0.023 and P=0.025, respectively).

Conclusions: Genetic variability of PD-1 and PD-L1 immune checkpoints may influence response to gemcitabine/cisplatin chemotherapy and could support personalized treatment in MM.

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P15.19C
Detection of cf*BRAF*^{V600E} in plasma from melanoma patients and non-melanoma individuals

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Approximately, 50% melanomas and 65% of benign nevi harbor the somatic alteration p.V600E in *BRAF* gene. Detection of this mutation in plasma (cf*BRAF*^{V600E}) is currently used for diagnosis and disease monitoring in melanoma. However, there are no information whether host factors may impact into cf*BRAF*^{V600E} detection. In this study, we quantified the cf*BRAF*^{V600E} by ddPCR in 211 individuals including 146 clinically confirmed non-melanoma individuals and 65 melanoma patients (25 stage III melanoma patients, 6 IV melanoma patients and 32 disease-free melanoma patients). Nevus count information and presence of clinically atypical nevi was available in 97.2% and 91.5% individuals, respectively. We detect cf*BRAF*^{V600E} in plasma of 83.3% of stage IV melanoma patient, 14.8% of stage III melanoma patients, 3.1% disease free melanoma patients and 4.1% of non-melanoma patients. The amount of cf*BRAF*^{V600E} plasma was higher in stage IV melanoma patients (377.8±5224 copies/mL) and stage III melanoma patients (16.7±214.8 copies/mL) compare to non-melanoma individuals (5.28±3.64 copies/mL). In addition, percentage of mutation differ between melanoma patients (>0.65% cf*BRAF*^{V600E}) and disease free melanoma patients and non-melanoma patients (<0.65% cf*BRAF*^{V600}). Association between nevus count or clinically atypical nevi and cf*BRAF*^{V600E} was not statistically assessed due to the limited number of cf*BRAF*^{V600E} subjects. However, individuals with high number of nevi showed higher concentration of cf*BRAF*^{V600E}. In conclusion, cf*BRAF*^{V600E} can be detected in individuals without melanoma. Thus, is necessary to stablish normality threshold of cf*BRAF*^{V600E} for the implementation of the analysis into clinical practice. This study was funded by Grant PI15/00956 from Fondo Investigaciones Sanitarias, Spain.

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P15.22B
Transcriptional modulation induced by fingolimod treatment in Relapsing Remitting Multiple Sclerosis patients

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Fingolimod (FTY) is a second-line drug approved for Relapsing Remitting Multiple Sclerosis (RRMS), known to prevent lymphocyte egress outside lymph nodes, thus reducing peripheral lymphocytes counts. We investigated transcriptional changes induced by the drug in immune cell

subtypes, to better elucidate its mechanism of action at the molecular and pathway levels.

Twenty-four RRMS patients were sampled at baseline and after 6 months of FTY treatment. CD3+ T cells and CD20+ B cells were sorted with MACS MicroBeads system and RNA sequencing performed using Illumina NextSeq500 platform. Differentially expressed genes (DEGs) were identified for each cell type using DESeq2 R package. Genes modulated by FTY (fold change [FC]>2 or FC<0.5 and false discovery rate [FDR]<5%) were considered for pathway analysis based on KEGG database. We observed a marked up-regulation in both T and B lymphocytes (313 up- and 240 down-regulated genes in T cells; 400 up- and 104 down-regulated genes in B cells), with evidence of significant overlap between the two cell types. Most of the DEGs had immune-related functions: among them, *CX3CR1* was strongly up-regulated ($p_{adjusted}=6.4x10^{-26}$, FC=5.96) and *CCR7* was down-regulated in both cell types ($p_{adjusted}=5.4x10^{-31}$, FC=0.18). DEGs were enriched of genes involved in immune-related pathways (e.g. Cytokine-cytokine Receptor Interaction, Chemokine Signaling Pathway). Network analysis elicited *CD44* as a hub gene involved in cell migration.

Our data suggest that fingolimod induces major transcriptional changes in genes with immune and cell migration functions; this modulation is shared between T and B lymphocytes.

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P15.24D

The evolving role of the clinical geneticist facing new technologies: the opinion of French clinical geneticists, molecular biologists and specialists, and the views of the European Reference Network ITHACA

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Over the previous decades, geneticists have had to adapt to the various technological and medical advances in their field. France is preparing for the arrival of next generation sequencing (NGS) in the care context, particularly for rare diseases and cancer. It was therefore mandatory for clinical geneticists (CG) to think about how they see their future role. A survey was distributed via Google Forms to 4 distinct groups of professionals. Overall, we received 126 questionnaires from CGs, 130 from molecular biologists, 172 from medical specialists, and 20 from ERN ITHACA coordinators. 60% of respondents think that only CGs should prescribe NGS tests, and even 100% for the prescription of exomes or genomes. Nevertheless, 80% think that a specialist who has acquired skills in genetics could prescribe gene panel analysis. The commonly raised issues were the limited time dedicated to the implications of test results, secondary data, management of VUS, and genetic counselling. It should be noted that CGs see this primary role in particular for polymalformative syndromes (75%), but less systematically for nonsyndromic intellectual disability (44%), single organ diseases in adults (retinitis pigmentosa 24%, MODY diabetes 6%). CGs are worried about the increase in the number of consultations, and the possible reduced quality of patient information if growing numbers of tests are prescribed by non-geneticists. These findings are compared with the results of the 3 other surveys, including the ERN, surveyed in order to learn how the transition had been managed in other European countries.

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P15.25A

Pharmacogenetic study of the nicotinic acetylcholine receptor subunit genes and smoking cessation among smokers receiving Varenicline treatment

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Introduction: Tobacco smoking is one of the major risk factors for many chronic diseases and is the leading cause of preventable deaths. Varenicline was approved by the FDA in 2006 as an aid to smoking cessation. However, not all the smokers are benefit from the efficacy of Varenicline. Only a few of pharmacogenetic studies were available and were primarily European Americans or of European origin. We aimed to assess cessation effect of nicotinic acetylcholine receptor subunit genes on smokers receiving treatment of Varenicline.

Methods: A total of 303 current smokers receiving Varenicline treatment were recruited from the smoking cessation clinics and were followed up for abstinence at 3, 6, 9, 12 months. Sixty-five single nucleotide polymorphisms on ten nicotinic acetylcholine receptor subunit genes were genotyped.

Results: We found that smokers carrying one additional G allele of *CHRNB4* rs1316971 had significantly higher probability of abstinence (OR=1.62, 95% C.I.: 1.08-2.42; OR=1.59, 95% C.I.: 1.06-2.39 at 6 or 9 month follow-up, respectively) when receiving Varenicline. Moreover, rs1316971 was also found to be associated with time to quit smoking (HR=1.33, 95% C.I.: 1.03-1.73). Besides, *CHRNA3* rs472054 (HR=1.93, 95% C.I.: 1.06-3.51) and rs578776 (HR=1.96, 95% C.I.: 1.09-3.51) were significantly associated with time to smoking relapse after quitting smoking.

Conclusions: Our study demonstrated that smokers carrying G allele of *CHRNB4* rs1316971 would quit smoking easily with Varenicline. This study was the first report of the association between nicotinic acetylcholine receptor subunit genes and smoking cessation with Varenicline treatment in Chinese population. Grant No.: NHRI-106-10304PI & NHRI-107-10304PI

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P15.26B

The homozygous Ile148Met Variant of PNPLA3 confers high risk for morbid obesity in a central european population

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p { margin-bottom: 0.1in; line-height: 120%; } Morbid obesity is a serious epidemic that is on the rise in all parts of the world. Roux-en-Y gastric bypass and modifications thereof enable short-term weight reductions and improve metabolic conditions in morbidly obese patients, but the response varies widely between individuals. We propose that these differences may in part be due to genetic differences. We studied a Central European population of morbidly obese patients who underwent gastric bypass (n=99). Our genetic study was performed using next-generation-sequencing (NGS) with a panel that included several genes and polymorphisms associated with obesity and other obesity-related phenotypes. One genetic polymorphism that stood out in particular was a single-nucleotide polymorphism (SNP, rs738409) located in the gene coding for the palatin-like phospholipase domain-containing protein 3 (PNPLA3, adiponutrin): one in three of the obese collective was a homozygous carrier of PNPLA3-148Met allele compared to one in thirteen in the general population. Interestingly, the number of heterozygous carriers was markedly lower (one in six vs. one in three) in the obese collective. Further, heterozygous carriers had difficulties sustaining their weight, one year post surgery. Our results demonstrate how a genetic variant can predict the long term benefit of a surgical procedure.

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P15.27C

Establishment of tumor-derived organoids: an approach to personalized medicine

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Introduction: Organoids are three-dimensional *in vitro* grown structures derived from induced pluripotent stem cells, adult stem cells or embryonic stem cells capable of self-renewal and self-organization, that exhibit the same organ functionality as the original tissue. They present organ-specific differential cells types and tissue compartmentalization. Additionally, they can be used to detect genetic alterations in patients. In this work we studied the potential of this type of culture for modeling cancer.

Material and Methods: Organoids from fresh tumor tissues of 40 patients with several types of cancer (colon (CRC), endometrial, ovary, kidney, lung, head and neck squamous cell carcinoma) were established. Tumor tissues were dissociated into functional units, seeded in Matrigel and cultured with the appropriate medium.

Results: We established organoids from six types of cancer and determined their culture conditions. The cytokines needed for each cell type were different but all of them include EGF, Noggin, Y-27632 and Wnt-3A. For organoids derived from CRC, R-spondin, FGF10, FGF2, prostaglandin E2, nicotinamide, A83-01 and gastrin are needed. Organoids derived from kidney cancer, also need VEGF. Those derived from lung cancer require VEGF and progesterone and β-estradiol are necessary for organoids derived from gynecological cancers.

Conclusions: Organoids can be obtained from different cancer tissues. For their development, it is essential to choose the right cytokines to grow them in. These cultures have shown to mimic three-dimensional structure of the origin tissue and could be a perfect source to determine genetic alterations in these patients.

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P15.28D

Identification of therapy-relevant "BRCAness"-associated candidate genes in ovarian cancer

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Introduction: The majority of patients with ovarian cancer is still diagnosed at an advanced stage of the disease generally leading to poor outcome. With PARP inhibitors being approved as a targeted therapy option for *BRCA*-mutated advanced ovarian cancer, the concept of "BRCAness" has become increasingly important. "BRCAness" describes the phenotypic characteristics that *BRCA1/2*-mutated tumors share with sporadic, non *BRCA1/2* mutated tumors, such as sensitivity to DNA double-strand break inducing agents. Our purpose was to search for other genes involved in homologous recombination repair that might be associated with "BRCAness" and/or better survival.

Material and Methods: We performed a qPCR-based mRNA expression analysis of six candidate genes in 48 ovarian cancer tissues. Optimal cut-off points for gene expression values were calculated with the Maximally Selected Rank Statistics in R. Overall survival (OS) was illustrated by Kaplan-Meier curves. Univariate and multivariate Cox regression analyses were performed with R.

Results: Patients with low mRNA expression of *BRCA1, BRIP1* and *RAD51C* showed significantly improved OS. Moreover, *BRIP1* gene expression persisted as an independent predictive factor for OS (HR: 4.38 [CI:1.56; 12.32], $p = 0.005$) in multivariate Cox regression analyses.

Discussion: Our data suggest a predictive role of *BRCA1, BRIP1* and *RAD51C* for the survival of ovarian cancer patients. Results may indicate that, besides *BRCA1/2*, patients with aberrant expression of *BRIP1* and *RAD51C* might also benefit from PARP inhibitors. Furthermore, they contribute to the understanding of the complex concept of "BRCAness". To confirm the results, candidate protein expression in tumor tissues needs to be explored.

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P15.29A

The effect of inflammation-related genetic polymorphisms on the occurrence of non-motor adverse events of dopaminergic treatment in Parkinson's disease

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Introduction: The role of inflammation in pathogenesis of Parkinson's disease (PD) is supported by candidate-gene and GWAS studies. It is widely accepted that pathways involved in disease pathogenesis may also influence treatment outcome. However, the influence of genetic variability in inflammatory pathways on treatment outcome has not been studied in PD, yet. Our aim was to investigate the association of selected single nucleotide polymorphisms (SNPs) in inflammatory pathways with non-motor adverse events (AEs) of dopaminergic treatment in PD.

Materials and Methods: We enrolled 223 PD patients and collected their demographic and clinical data including non-motor AEs (nausea/vomiting, orthostatic hypotension, peripheral oedema, sleep attacks, visual hallucinations, and impulse control disorders). DNA was isolated from peripheral blood samples and genotyped for *NLRP3* rs35829419, *CARD8* rs2043211, *IL1β* rs16944, *IL1β* rs1143623, *IL6* rs1800795, and *TNFα* rs1800629. Statistical analysis using logistic regression was performed.

Results: The study included 58% males and 42% females, with median age at PD onset 62 (55-71) years and median disease duration 7.5 (4-14) years. *IL1β* rs1143623 (c.-1591G>C) was associated with occurrence of orthostatic hypotension (OH). Heterozygotes had a lower chance of developing OH ($p = 0.028$; OR=0.510; 95% CI=0.279-0.931) compared to homozygotes for wild-type allele. This association remained significant after adjusting for gender and disease duration ($p = 0.026$; OR=0.500; 95% CI=0.272-0.920). No other SNP showed any association with other studied AEs.

Conclusions: Our pilot study indicates a link between inflammatory pathways and OH as AE of dopaminergic treatment in PD. Further studies are needed to explain the detected association.

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P15.30B

The Undiagnosed Rare Disease Program of Catalonia (URDCat) - illustrating the value of NGS for personalised medicine

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Almost 40 million people in Europe are affected by one of the ~7000 rare diseases (RDs). Unfortunately, a significant percentage of patients remain undiagnosed after years of investigation, representing a real burden to the health care system.

The URDCat (Undiagnosed Rare Disease Program of Catalonia) project was launched in 2017 and aims to enable the Catalan Health System to provide personalised genomic medicine as a fully integrated service for patients with RDs, initially as a pilot project for RDs with neurologic involvement. URDCat comprises a collaboration between 16 groups, associated with 7 hospitals across Catalonia.

The project has several overarching objectives: (i) standardise the process of analysis and integration of clinical and genomic data; (ii) implement a platform for analysis of genomics data that is suitable for clinical practice; (iii) identify causative genetic variants of undiagnosed RD patients; (iv) highlight the utility of genomics, and advance its usage as a tool of personalised medicine for RD diagnostics and (v) educate stakeholders in the value of genomic data.

Deep phenotyping data has been collated from more than 750 families with a customised PhenoTips form using HPO, ORDO and OMIM. Over 220 associated genomic datasets have been re-analysed in detail using the URDCat platform, based on RD-Connect. Pathogenic or candidate causative variants have been identified for almost 20% of the re-analysed cases, which are currently being followed up in the laboratory. In order to solve remaining cases, whole exome or genome sequencing has been undertaken for around 500 prioritised index cases.

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P15.31C
Genotyping approach supporting pre-emptive pharmacogenomics testing: The Slovenian experience within the UPGx project

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Introduction: The Horizon 2020 UPGx (Ubiquitous Pharmacogenomics: www.upgx.eu) project aims to make actionable pharmacogenomics data and effective treatment optimization accessible to every European citizen in order to improve patient treatment outcome and consequently lower the expenses of the treatment. According to the existing data published and Dutch Pharmacogenetics Working Group (https://www.pharmgkb.org/page/dpwg), the guidelines made by the UPGx Consortium revealed that actionable drug prescribing for 55 drugs is most strongly linked to 13 pharmacogenes (www.pharmgkb.org/cpic/pairs). One of the aims of the project was to implement genetic testing for a panel of pharmacogenes to support a clinical study on pre-emptive pharmacogenomic testing in seven European countries, including Slovenia.

Methods: 1.Setting up a panel of common functional variants within pharmacogenes with major influence on interpatient variability in drug response and existing evidence based treatment guidelines. 2.Setting up the infrastructure and validation of methodology for prospective genotyping for selected variants.

Results: In total 48 common functional SNPs along with the *CYP2D6* deletion and duplication were identified as targets for pharmacogenetics testing. Real-time PCR fluorescence-based endpoint genotyping (KASPar) was chosen as the genotyping method and SNPline (LCG Group) genotyping system was set up at the Pharmacogenetics Laboratory. The respective KASPar assays were designed by LCG Group and Biologis and introduced, validated and tested at the Pharmacogenetics Laboratory. LR-PCR amplification followed by SNP genotyping was used to detect *CYP2D6* polymorphisms.

Conclusions: We have successfully implemented the genotyping methodology and infrastructure for pre-emptive pharmacogenomics testing within the UPGx Project.

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P15.33APharmacogenetics of Eliglustat in Gaucher disease

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Introduction: In 2015 European Union approved Eliglustat as first-line oral treatment for adult patient with type 1 Gaucher disease. It is important for these patients to genotype the gene that encode the main metabolizer enzyme of this drug (CYP2D6). The clinical trials of Eliglustat shows that the concomitant use of this drug with CYP3A inducers is not recommended in this patients. For this reason, it is also important to know the genotype of the other genes that are involved in metabolism of this drug such as *CYP3A4* and *ABCB1* genes.

Material and Methods: DNA samples of 61 Spanish patients with type 1 Gaucher disease were genotyped using the xTAG® CYP2D6 kit v3 protocol for *CYP2D6* gene, PCR- RFLP for *CYP3A4*1B* and *ABCB1* haplotype, and RT-PCR for *CYP3A4*22*.

Results: The metabolizer status of CYP2D6 was 4.9%, 13.1% and 82% for poor, intermediate and extensive, respectively. The genotype of *CYP3A4*22* showed that 94.8% of patients were wild-type for this SNP and 5.2% heterozygous. The SNPs of *ABCB1* gene (rs1045642, rs2032582, rs1128503) related with low expression of P-glyprotein are infrequent in our cohort. This is important because it prevents the eliglustat accumulation in the brain.

Conclusions: In the patients who have ultra-rapid genotype for *CYP2D6*, the use of Eliglustat is not approved. For the rest of patients it is important to have genotyping information about *CYP3A4* and *ABCB1* and to assess concomitant use of medications and herbal supplements to ensure a precision personalized medicine.

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P15.34B
Pharmacogenetics of everolimus in the treatment of the rare disease tuberous sclerosis

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Introduction: Everolimus is an immunosuppressant recently accepted to treat adjacent symptoms to the rare disease tuberous sclerosis (or Bourneville syndrome), as renal angiomyolipoma and giant cell astrocytoma. The prevalence of this pathology is between 1:6000 and 1:10000. Most genes have shown to be close relationated with pharmacokinetics and pharmacodynamics of everolimus, so their polymorphisms can cause a great interindividual variability in its plasmatic levels. The narrow therapeutic window of this drug generates the risk of occurrence adverse drug effects or lack of effectiveness. The Single Nucleotide Polymorphisms (SNP) analyzed to study its genotype-phenotype relation are *CYP3A5*3* (6986 A>G), *CYP3A4*1B* (g-290 A>G), *CYP3A4*22* (15389 C>T), *ABCB1* (c.1236C>T), *ABCB1* (c.2677G>T), *ABCB1* (c.3435C>T), *PXR* (c.44477T>C), *PXR* (c.63396C>T), *PXR* (c.69789A>G), *CYP2C8*3* (416 G>A), *CYP2C8*3'* (1196 A>G) and *CYP2C8*4* (792 C>G).

Material and Methods: A total of 8 voluntary patients who suffer tuberous sclerosis in treatment with everolimus participate in this study. Blood samples were collected in FTA^{TM}cards. RFLP-PCR has been performed to genotype the selected SNPs, being *CYP3A4*22* genotyped by RT-PCR.

Results: The TTT haplotype of A*BCB1*, *CYP3A5*3*, *CYP2C8*3*, *CYP2C8*4* and *CYP3A4*22* would increase the drug levels and thus produce a higher risk of adverse effects. On the other hand, the *CYP3A4*1B* and *PXR* SNPs would decrease drug levels and thus produce lack of therapeutic effectiveness.

Conclusions: The genotyping of patients treated with everolimus is a useful tool to predict and explain interindividual dose variations and thus offer relevant information to improve the treatment of patients.

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P15.35B
Strengthening pharmacogenetics

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Introduction: Pharmacogenetics is a relatively new science that has not been studied widely in health sciences degree programmes. Nevertheless, it is considered as a tool that contributes to precision medicine since it provides relevant information about the patients and could predict the effectiveness of the drugs or the occurrence of adverse drug reactions. Health Sciences professionals, mainly the pharmacists, should know the clinical applications of this area of knowledge or at least they should understand its main concepts and basic vocabulary. For this reason, the first aim of this project was to develop a guideline about Pharmacogenetics for pharmacists who have not received any formation about it.

Materials and Methods: Pharmacy students of the subject "Pharmacogenetics and Pharmacogenomics" have selected the most important information about the different sections of the guideline: glossary of terms, basic concepts about pharmacokinetics and pharmacodynamics, main genes involved in metabolism, transport and receptors of drugs with pharmacogenetic relevance and frequently asked questions. The content is revised and corrected by Pharmacy students of "Pharmacology" and "Pharmacokinetics" subjects. In order to get an attractive aspect of the guideline, Architecture students of "Analysis of Architectonic forms" develop its graphic design.

Results: After the edition period, the guidelines will be distributed to the different pharmacies of the area close to San Jorge University. Its usefulness will be tested among them.

Conclusions: It is hoped that through this activity, knowledge about pharmacogenetics will be accessible for pharmacists so that this science could be more understood.

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P15.36D
Pharmacogenomic recommendations based on different sequencing and genotyping platforms: challenges and solutions for using existing guidelines

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Introduction: The past decade has shown an increase in the amount of genetic information that could be translated into clinically actionable decisions tailoring medical therapy. The Clinical Pharmacogenetics Implementation Consortium has prepared curated genotype-based guidelines for translating genetic information into drug prescription recommendations for 14 genes associated with 36 different drugs. Here, we investigated how successfully we could translate existing genotype data into informative recommendations.

Materials and Methods: We surveyed the genotype data of 44 448 Estonian Biobank participants: 2420 with whole genome sequence (WGS), 2445 with whole exome sequencing (WES), 8132 genotyped on the HumanOmniExpress beadchip (OMNI), and 33157 on the Global Screening Array (GSA) from Illumina. The genotype data obtained on both arrays were phased and imputed. For detecting star alleles, we used gene-specific star allele definition tables from the PharmGKB database.

Results: The greatest challenge that we faced was large allele definition tables that do not appear to cover all possible allelic combinations, and limited guidance on how to prioritize the variants in cases of multiple or no matching star alleles. When comparing the proportion of multiple matching star alleles, WGS clearly stands out with 2.8% ambiguous calls, followed by 32% for GSA, 35% for OMNI and 61% for WES as several important variants fall outside the coding region of the genome.

Conclusions: Translating existing genotype and WGS data into pharmacogenomic recommendations for biobank participants illustrates the importance and innovative potential of curated guidelines: a crucial step in advancing the whole process of personalised medicine in general.

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P15.37A

The prevalence of predicted drug-gene-interactions in 12.792 individuals from the Dutch Lifelines Cohort

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Introduction: (Adverse) effects of drug treatment for similar indications can show striking interindividual differences. Despite the evidence for pharmacogenomic (PGx) tests to provide precision medicine, clinical use is still limited. We conducted a study on the prevalence of drug-gene-interactions (DGIs) in the Dutch Lifelines Cohort (www.lifelines.nl) and are exploring the practical barriers and success factors of pre-emptive PGx-testing in a clinical pilot.

Methods: Genotypes in Lifelines were identified using Illumina CytoSNP12 array data and GoNL (www.nlgenome.nl) imputed, translated to PGx star-alleles and predicted phenotypes following national PGx guidelines. Due to the absence of copy number variance (CNV), genotypes for CYP2D6 were not available. DGIs were estimated using Lifelines questionnaire's medication history. In addition to the Lifelines study, a clinical pilot (n=300) is being conducted in our hospital's outpatient clinics. The prevalence of DGIs will also be assessed for this clinical cohort.

Results: 12.792 individuals, 95,5% of the genotyped Lifelines Cohort, have at least one clinically relevant PGx variant. Prescription drug use was linked to the genetic data in 6.415 (47,9%) individuals where 435 DGIs were identified amongst 420 individuals (6,55%). We observed similar prevalence in the preliminary data of the clinical cohort.

Conclusions: PGx variants and estimated DGIs are abundantly present in the Lifelines Cohort. Prevalence is expected to become even higher when CYP2D6 genotypes are taken into account and when DGIs are identified based on drug prescription data which will become available in the near future.

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P15.38B

SLCO1B1 c.521T>C genotype, sex, and initial statin treatment as contributing factors to continuous simvastatin or atorvastatin treatment in a Dutch cohort

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Introduction: Statins reduce low-density-lipoprotein cholesterol levels and lower the risk for cardiovascular events. Although statins are generally well-tolerated, patients can experience adverse drug events (ADE), and discontinue their treatment. In this study we aim to investigate if and how pre-emptive pharmacogenomic *SLCO1B1* c.521T>C screening would have influenced the statin treatment outcomes for patients in primary care.

Methods: The amount of ADE experienced by 1159 patients treated with statins in a Dutch cohort were measured through three proxies: mean dose change, discontinuing treatment or switching statins and, time to establish stable dosing. Information on their statin use was evaluated. For every outcome, patients were compared on their *SLCO1B1* genotype, sex, and type of statin. Analyses were corrected for relevant confounders.

Results: At baseline, female simvastatin users received a lower dose compared to male simvastatin users. Regardless of determinants, approximately half of patients discontinued their statin treatment within three years follow-up of starting their treatment. The proxies for ADE showed no statistically significant differences between *SLCO1B1* genotypes TT versus TC and CC, men versus women or simvastatin versus atorvastatin.

Conclusions: The results indicate that *SLCO1B1* c.521T>C screening would not have contributed to less discontinuation and switches in simvastatin and atorvastatin prescription for patients in our Dutch regional cohort. Factors that do contribute to approximately half of patients discontinuing their statin treatment should be studied further to gain insight in determinants of unsuccessful implementation of statin treatment.*Grant: RIVM (S/132001/01/PD), Project: "Personalised medicine: eligible or not?" about eligiblility of pharmacogenomics in primary care*

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P15.39C

New horizons for Prader-Willi patient's assistance: families' centered health care

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Background: Prader-Willi Syndrome (PWS) is a genetic condition with recognizable pattern of physical findings with significant neurocognitive, endocrine and behavioral abnormalities. It is caused by lack of expression of genes from an imprinted region of the paternally inherited chromosome 15q11-q13 region. We aim to improve the quality of life and healthcare of patients and families with PWS.

Method: PWS patients under 18 had been coordinated in SJD-Hospital between March 2016 and December 2017. Multidisciplinary medical following is needed due to the multisystemic involvement. American Academy of Pediatric recommendations were followed and coordinated by a Case Manager (CM). We assess patients' experience, consultation adherence and extraprotocolized needs.

Results: 37 patients were attended coordinately 2-3 times per year at the hospital, thanks to the consecutive-concentrated visits and scheduled coordination of the CM. Lost days of work and school absences were reduced. Meetings with all the involved physicians to discuss the best personalized therapeutic attitudes were scheduled at the end of the day. CM and the Social Worker were also included. The degree of satisfaction of the family increased in all cases.

Conclusions: The PWS functional plan improves the patient's care. CM plays an essential role to coordinate the assistance of each patient to interfere as less as possible in their lives: 1) grouping consultations to the hospital avoiding unnecessary journeys, 2) coordinating with other areas such as school or primary care to ensure continuity of care and 3) collaborating with other specialties for a global management of the patient and their families.

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P15.40D

PREemptive Pharmacogenomic testing for Preventing Adverse drug Reactions (PREPARE) study: The Slovenian Experience within the UPGx project

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Introduction: The clinical study PREPARE within the Horizon2020 Ubiquitous Pharmacogenomics (U-PGx) project (www.upgx.eu) aims to establish if implementation of PGx-guided drug prescribing for a panel of drug-pharmacogene pairs reduces drug-genotype associated adverse drug reactions (ADRs) in comparison to patients receiving standard of care treatment in seven European countries, including Slovenia.

Methods and Results: Within a prospective, block-randomized, controlled clinical study, Slovenia was randomized to start with PGx-guided prescribing (study arm) and will switch to standard of care (control arm) after 18 months. Up to now, 150 patients participated in the study. They were invited when having a drug with DPWG guideline (index drug) first prescribed in the routine care. DNA samples from patients were genotyped for a panel of 50 genetic variants in 13 pharmacogenes. Within 3-5 working days genotype data and DPWG guidelines were available to physicians to modify the treatment with the index drug if recommended. Treatment outcomes were followed for 12 weeks. The most commonly prescribed index drugs were statins (40%), tacrolimus (20%) and antidepressants (23%). The number of polymorphic pharmacogenes was from 1 to 8 per patient, with the average of 4.2. In total 74 patients (50%) carried polymorphic *CYP2D6*, which is the gene with the highest number of therapeutic recommendations. In 30.5% of patients the therapeutic recommendations included the index drug.

Conclusions: Within the U-PGx project and the PREPARE study panel-based pre-emptive pharmacogenomic was successfully introduced in Slovenia.

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P15.41A

800 exomes for rare disease research: outcomes of the transnational BBMRI-LPC WES call in collaboration with EuroBioBank and RD-Connect

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The 2016 BBMRI-LPC WES Call offered a unique free-of-charge opportunity to genetically diagnose rare disease patients with biological samples deposited within the EuroBioBank network. 800 whole exomes were sequenced from 17 distinct projects, each having 2-3 principal investigators from different countries. The projects spanned a wide range of rare disease phenotypes, including neuromuscular disorders, inborn errors of metabolism, albinism, and sudden cardiac depth, amongst others, and informed consent had to permit data sharing for research purposes through controlled access repositories such as the EGA (https://ega.crg.eu/) and RD-Connect (http://rd-connect.eu/). Clinical and phenotypic information for every case was collected in RD-Connect's customised PhenoTips instance, using standards such as the Human Phenotype Ontology (HPO), OMIM and Orpha codes, and sequencing undertaken at the CNAG in Spain, and the WTSI in the UK. Sequencing data was processed using the RD-Connect standard analysis pipeline and results made available through the RD-Connect Genome-Phenome Analysis Platform (https://platform.rd-connect.eu/) once all call requirements had been met. The Platform allows researchers to analyse and interpret their genotype:phenotype data privately for up to 6 months before it is shared with other authorised users. It also facilitates anonymised data sharing through APIs from initiatives such as the GA4GH/IRDiRC MatchMaker Exchange (http://www.matchmakerexchange.org/) and Beacon Network (https://beacon-network.org). We report on the challenges and lessons learned from conducting such a complex transnational collaborative initiative and present an up-to-date diagnostic yield of the project as a whole, with some illustrative success stories.

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P15.42B

Novel candidate gene associated with mTOR inhibitor response in Renal Cell Carcinoma

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Introduction: Inhibitors of the mammalian target of rapamycin (mTORi) are used to treat several cancers, including renal cell carcinoma (RCC). Although response to these drugs can be partially explained by mutations activating mTOR pathway, the majority of responders have no mutations identified. This suggests that additional genes may contribute to mTORi response. In this study, genomic screening of chromophobe RCC (chRCC) patients sensitive to mTORi, together with *in vitro* functional studies, identified a novel candidate predictive gene.

Materials and Methods: Whole exome sequencing (WES) was applied to three chRCC patients sensitive to mTORi. An independent chRCC series was collected for targeted sequencing and immunohistochemistry (IHC) of candidate genes. Rapamycin sensitivity and mTOR pathway activation was assessed in cells with shRNA-mediated gene silencing.

Results: WES revealed one single mutated gene, not related to mTOR pathway, shared by the three chRCC tumors. Two of the somatic mutations were loss-of-function, one was a misssense variant. IHC staining and Sanger sequencing validated the results. Inactivation of the gene was detected at low frequency in an independent chRCC cohort (3 out of 96 tumors), in agreement with TCGA (2 out of 66). MTT assays revealed that depletion of the protein through shRNAs, or treatment with an specific small-molecule inhibitor, sensitized HeLa cells to rapamycin. mTOR pathway activation, measured by p-S6/p-S6K1/p-AKT, was not altered in shRNA-treated cells, suggesting mTORi sensitizing effects occurred through alternative effectors.

Conclusions: We propose a novel candidate gene associated with mTORi response, which may help to select patients sensitive to mTORi therapy.

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P15.43C

Framework for clinical assessment of Mendelian inherited risk alleles

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There is an increased interest and demand in clinical genomics to interpret and return Mendelian-inherited risk alleles to the electronic medical records. Although this is emerging as part of routine clinical practice, there is no formal terminology nor a framework for classification of these low-penetrant variants. Therefore, we established an evidence-based framework to clinically classify risk alleles into three categories recommended by ACMG: established risk allele, likely risk allele, and uncertain risk allele. Evidence used for risk allele classification was collected by a thorough literature search for case/control studies, meta-analyses, functional data, and included cohort size, phenotyping, statistical significance, and replication of results over time. For example, to be classified as an established risk allele, a variant must meet the following criteria: 1) significant results after Bonferroni, false discovery rate or permutation correction; 2) odds ratio > 2; 3) results replicated in at least 5 studies by different laboratories using underlying cohorts, including through meta-analyses. To test this method, we classified seven well-established risk alleles all of which met our criteria for established risk variant. In addition, we classified five previously reported risk alleles. Of these, two were established risk alleles, one was likely risk allele, and two were uncertain risk alleles. This framework establishes a standardized approach to

classifying risk variants with high individual odds ratios and provides clinicians and patients with important, contextualizable information. This process will be incorporated into our laboratory for additional risk allele interpretations and will be extended to a similar framework for protective alleles.

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Guidelines for reporting secondary findings of genome sequencing in cancer genes: the SFMPP recommendations

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Large-scale genomic sequencing for clinical purposes has led to an increase discovery of secondary findings (SF). The increasing use of multi-gene panel analysis to explore familial cancer predisposition and of tumor genome analysis rise important questions regarding SF in cancer susceptibility genes. The American College of Medical Genetics and Genomics (ACMG) published a policy statement for SF management for a list of genes including 29 cancer genes. There are currently no equivalent recommendations in Europe. From June 2016 to May 2017, the French Society of Predictive and Personalized Medicine (SFMPP) establish a working group included 47 experts to elaborate guidelines about management of SF in cancer genes presented here. A first workgroup including ethicists, lawyers, representatives of patients' associations, and psychologists, provides ethical reflection, information guidelines and information materials (written consent form and video). A second group dedicated to medical expertise including oncologists, clinical and molecular geneticists provides independent evaluation and classification of 60 genes. The main criterions were the "actionability" (access to validate screening or prevention strategies), the risk evaluation (severity, penetrance, and disease's age of onset), and the level of evidence from published data. Genes were divided into 3 subgroups: class 1 for which it is recommended to return the results (n=28), class 2 for which the restitution remains questionable (n=18) and class 3 for which it is not recommended to return the result (n=14). These first European guidelines on SF in cancer predisposing genes may help clinicians and laboratories to standardize and guide practices.

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P15.45A

Secondary findings on multigene panels: A new frontier for clinical utility in hereditary cancer genes

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Medically actionable secondary findings in whole exome/genome sequencing (WES/WGS) are reported independent of WES/WGS indication. Multigene panels can cover 100s of genes and reveal hereditary cancer gene secondary findings. We report the prevalence of secondary findings identified via multigene panel in 3000 individuals of multiple ethnicities undergoing cardiovascular genetic testing and the clinical utility of these cancer-risk gene findings.We analyzed de-identified data for 47 cancer-risk genes in 3679 patients referred for hereditary cardiovascular genetic testing. Frameshift, nonsense, and splice-site disruptions were classified as pathogenic variants (PV).We observed 141 PVs in cancer-risk genes, for a prevalence of 6.03%, including PVs with established management guidelines in *ATM, BRCA1, BRCA2, CHEK2, MUTYH, PALB2, PMS2*. The prevalence of secondary findings by ethnicity in our cohort was: African American (1.73%), Asian (8%), Hispanic (4.98%), Caucasian (6.43%).Multigene panel cancer-risk secondary findings prevalence is higher than studies using WES/WGS. The distribution of findings by ethnicity suggests pathogenic missense variants from lower prevalence populations are underrepresented in current databases, and the true prevalence of secondary findings is underreported. Follow-on analysis assessed the clinical utility of cancer-risk gene panels in breast cancer patients. In 80% of breast cancer cases clinical management for patients and/or their family members was changed based on PVs in *ATM, CHEK2, MUTYH, PALB2, PMS2* and others, impacting the outcome of almost half of patients. These data support the clinical utility of PVs in cancer-risk genes and suggest a role for multigene panels in the detection of clinically actionable secondary findings.

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P15.46B
Novel approach enables rapid deployment of high quality, flexible content target enrichment panels

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Efficient utilization of targeted gene panels for clinical research is challenged by the wide variation in gene constituents specific to a given study. While focused gene panels efficiently provide the necessary depth of coverage for low frequency variant detection, the high costs and technical requirements associated with panel design present challenges. The NEBNext Direct technology utilizes a novel approach to selectively enrich nucleic acid targets ranging from a single gene to several hundred genes, without sacrificing specificity. The approach rapidly hybridizes both strands of genomic DNA with biotinylated probes prior to streptavidin bead capture, enzymatic removal of off-target sequences, and conversion of captured molecules into sequence-ready libraries. This results in an exceptionally uniform coverage profile for a given target. Unlike alternative hybridization methods, this 1-day workflow does not necessitate upfront library preparation, and instead converts the captured molecules into libraries compatible with Illumina sequencing. We have designed and optimized baits specific to the full exonic content of a growing library genes associated with cancer, neurological disorders, autism, cardiac disease, and other conditions. These are designed, balanced, and pooled on a per gene basis, and can be combined into customized panels, allowing rapid turnaround of specific custom gene subsets. Here, we present the ability to rapidly deploy custom gene panels across a variety of panel sizes and content, while maintaining high specificity, uniformity of coverage across target content, and sensitivity to detect nucleic acid variants from clinically relevant samples.

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P15.47C
Targeted high-throughput sequencing in thyroid cancer diagnostics

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Introduction: Identification of driver and secondary mutations with targeted high-throughput sequencing is a promising approach in diagnosis and management of thyroid cancer.

Materials and Methods: Point mutations, fusions and CNV known to be associated with sporadic thyroid cancer were selected from COSMIC and TCGA project data. Additionally, exons of *RET* gene harboring germline mutations were included. Design of primers for targeted amplification of regions with pathogenic point mutations was done with AmpliSeq Designer v. 5.4.1, the length of amplicons was set in range of 125-375 b.p. The technical validation was performed on cell lines SW620, MCF7. FNA samples of papillary and follicular carcinomas (15 samples) and surgical samples of normal thyroid tissue (11 samples) were analyzed. Analytical sensitivity was assessed via introduction of DNA with known mutations into DNA of normal tissue, which did not harbor pathogenic mutations.

Results: The design included over 450 point mutations in 25 genes, 3 regions of CNVs and 25 types of fusions. Mutations expected in cell lines (according COSMIC CLP and Cancer Cell Line Encyclopedia) were detected: *APC* p.Q1338* and *KRAS* p.G12V – in SW620; *PIK3CA* p.E545K – in MCF7. The panel is highly sensitive and successfully identifies as low as 3% of alleles. In PTC samples following mutations were detected: *BRAF* p.V600E (5 samples); *KRAS* p.G12V; fusions *ETV6-NTRK3*, *PAX8-PPARG*, *NCOA4-RET*. In the sample with ETV6-NTRK3, secondary mutation *IDH1* p.V178I was found. Supported by The Foundation for Assistance to Small Innovative Enterprises in Science and Technology (№ 442ГС2/9119 - С3-40846).

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P15.48D

Observation of wide phenotypic variability in 3 individuals with the same pathogenic mutation in *PAX3*

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Introduction: The *PAX3* gene encodes an important transcription factor regulating the expression of many genes and playing a crucial role during foetal development. Pathogenic mutations in this gene lead to autosomal dominant forms of Waardenburg syndrome and Craniofacial-deafness-hand syndrome.

Methods and Materials: A 29-year-old male diagnosed with Waardenburg syndrome, who exhibited unilateral deafness, pigmentation changes and above-average intelligence, and a 5-year-old girl with moderate mental retardation of unknown cause, speech delay and light skin, hair and eyes were tested with the TruSight One gene panel on a MiSeq platform. Consequently, the younger brother of the WS patient, who had bilateral deafness, pigmentation changes, CNS malformations and borderline IQ, was tested by targeted amplicon NGS sequencing for the mutation detected in his relative. The family of the older brother subsequently decided to undergo preimplantation genetic diagnosis. Four day-3 embryos were biopsied and tested for carrier status of the mutation through targeted NGS.

Results: Despite their wide phenotypic variability all three patients were found to be heterozygous carriers of the same non-sense mutation in *PAX3*: Chr2:g.223160283T>A, NM_181459.3:c.415A>T, NP_852124.1:p.Lys139Ter. Performing PGD we established that two of the four embryos tested were not carriers and were suitable for transfer.

Conclusions: Variable expressivity complicates not only the clinical identification of a particular syndrome and subtype, but also makes genetic counselling in regard to prenatal diagnosis challenging and risky. Performing PGD gives the opportunity to avoid the moral dilemma whether to keep the embryo in conditions where the phenotype could vary from debilitating to less severe.

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P15.49A

Whole Genome Sequencing in healthy people

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Introduction: Whole genome sequencing (WGS) provides the most comprehensive collection of an individual's genetic variations compared to the human reference genome. WGS can be a useful diagnostic and research tool, as it allows the detection of pathogenic and susceptibility variants enabling the diagnosis of an associated syndrome as well as the identification of variants explaining the observed genetic diversity.

Purpose: The purpose of this study was to evaluate the clinical significance of WGS in healthy people regarding the detection of mutations associated with clinical disorders,

predisposition to various clinical conditions and response to drugs.

Materials/Methods: 50 healthy people were subjected to WGS conducted on BGISEQ-500 sequencing platform which offers high quality data with average coverage 30x. The vcf files were filtered based on the following exclusive criteria MAF≥1% and assignment by ClinVar as benign/ likely benign.

Results: Several mutations with clinical implications were identified in all healthy participants. Table 1 depicts some of our findings.

TABLE 1:

SAMPLE ID	GENE/ MUTATION	
25008	CFTR:c.1521_1523delCTT	Cystic fibrosis (INHERITED)
25972	CYP4F2:c.1297G>A	DRUG RELATED
25987	MEFV:c.2082G>A	Mediterranian syndrome (INHERITED)
30007	BCHE:c.1256G>T	DRUG RELATED
30125	BRCA1:c.181T>G	PREDISPOSITION to cancer
30982	MYH7:c.3645G>C	PREDISPOSITION to familial hypertrophic cardiomyopathy
30999	DYSF:c.386G>A	"Miyoshi myopathy" (INHERITED)
32039	CFTR:c.489+1G>T	Cystic fibrosis (INHERITED)
31033	GJB2: c.35delG	Nonsyndromic hearing loss (INHERITED)
35000	HBB:c.93-21G>A	Beta thalassemia (INHERITED)
35022	BRCA2: c.3554_3563delCAGTTGAAAT	PREDISPOSITION to cancer
31025	ABCA4:c.2690C>T	Stargart macular degeneration (INHERITED)
35064	BRCA2:c.5722_5723delCT	PREDISPOSITION to cancer

Conclusions: WGS should be the method of choice not only in cases with designated phenotype but also in the context of carrier screening.

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P15.50B

Pharmacogenetic study of seven polymorphisms in three nicotinic acetylcholine receptor subunits in smoking-cessation therapies

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Introduction: Smoking-cessation therapy reduces the risk of smoking-related diseases, but is successful only in a fraction of smokers. There is growing evidence that genetic variations in nicotinic acetylcholine receptor (nAChR) subunits influence the risk of nicotine dependence and the ability to quit smoking.

Materials and Methods: 337 smokers who underwent pharmacotherapy with varenicline, bupropion, nicotine replacement therapy (NRT) alone, or NRT plus bupropion were genotyped for 7 germline polymorphisms in genes encoding the nAChR subunits *CHRNA4*, *CHRNA5*, and *CHRNB2* by pyrosequencing. Smoking habit and abstention were assessed from the number of cigarettes smoked per day (CPD) and the exhaled CO (eCO), at four time points (baseline, 1, 3 and 12 months). Statistical tests were carried out with PLINK software using sex, therapy, and eCO as covariates. Significance was set at $P < 0.05$. Multiple testing was done using the Benjamini-Hochberg procedure.

Results: At baseline, both CPD and eCO were associated with polymorphisms in the *CHRNA5* locus (rs503464, rs55853698, rs55781567 and rs16969968; $P < 0.01$). rs503464, a variant in the 5′-UTR of *CHRNA5*, was also associated with 1-, 3- and 12-month responses to therapy ($P = 0.011$, $P = 0.0043$, $P = 0.020$, respectively), although after correction for multiple testing only the association at the 3-month assessment remained significant.

Conclusions: These data support the role of individual genetic makeup in the ability to quit smoking.

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P16 Omics/Bioinformatics

P16.01A

3DIV : A 3D-genome Interaction Viewer and database

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3DIV: A 3D-genome Interaction Viewer and database

Abstract: Three-dimensional (3D) chromatin structure is an emerging paradigm for understanding gene regulation mechanisms. Hi-C (high-throughput chromatin conformation capture), a method to detect long-range chromatin interactions, allows extensive genome-wide investigation of

3D chromatin structure. However, broad application of Hi-C data have been hindered by the level of complexity in processing Hi-C data and the large size of raw sequencing data. In order to overcome these limitations, we constructed a database named 3DIV (a 3D-genome Interaction Viewer and database) that provides a list of long-range chromatin interaction partners for the queried locus with genomic and epigenomic annotations. 3DIV is the first of its kind to collect all publicly available human Hi-C data to provide 66 billion uniformly processed raw Hi-C read pairs obtained from 80 different human cell/tissue types. In contrast to other databases, 3DIV uniquely provides normalized chromatin interaction frequencies against genomic distance dependent background signals and a dynamic browsing visualization tool for the listed interactions, which could greatly advance the interpretation of chromatin interactions. '3DIV' is available at http://kobic.kr/3div.

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P16.02B

Improved accuracy of ultra-low frequency mutation detection by novel duplex sequencing adapters and consensus read reconstruction

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Detection of low to ultra-low frequency mutations, enabled by advances in next generation sequencing (NGS), is often confounded by errors introduced during standard NGS workflows, even those using unique-molecular-identifiers (UMIs). We have developed xGen® Duplex Seq Adapters, which permit double-stranded DNA tagging and consensus read calling among duplicated sequences. These novel adapters are compatible with standard library preparation and enrichment methods, yet enable duplex sequencing and enhanced error correction. Well-established genomes, or commercially acquired and individually genotyped FFPE and cfDNA samples, were mixed to mimic low-frequency variant samples with MAF down to 0.1%. Libraries from these sample mixtures were prepared for deep sequencing using Duplex Seq Adapters and a 75 kb custom, target-capture panel. A bioinformatic tool suite was developed to create UMI-aware consensus reads. Compared to standard libraries, libraries made using the Duplex Seq Adapters, with their unique tagging structure, had comparable or better yields and mean deduplicated sequencing depth for all sample types and input masses tested. For variant calling accuracy, evaluated against published cell-line or genotyped ground truths, Duplex Seq Adapters offered >30-50 fold better specificity than standard adapters at the same level of detection sensitivity, regardless of sample type. For example, using Duplex Seq Adapters paired with consensus read construction, >90% detection sensitivity was achieved for genomic and cfDNA samples at 0.5% MAF with 0 false positives, resulting in 100% specificity and >40-fold error suppression compared to standard adapters. Such advances in detection accuracy are key to refining diagnostics and improving precision cancer care.

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P16.03C

Comprehensive map of regulated cell death signalling network: a powerful analytical tool for studying diseases

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Introduction: The Cell death process draws special attention, due to frequent perturbations of its machinery in various diseases. It can be described into three highly interconnected steps: initiation, signaling and execution of death signals.

Materials and Methods: We used a systems biology approach to graphically represent biological processes in a seamless comprehensive map of signaling network that is accessible for visualization, computational analysis and applications.

Results: The Regulated Cell Death (RCD) map is divided into 27 functional modules that can be visualized

contextually in the whole map, or as individual diagrams. The map contains more than 1200 proteins and genes, 2020 biochemical reactions and is based on 600 scientific papers. It is an open source platform facilitated by the NaviCell web tool for map navigation (https://navicell.curie.fr/pages/maps_rcd.html). The RCD map was used for functional interpretation of the differences in cell death regulation between Alzheimer's disease and lung cancer. These diseases were demonstrated to have a reverse comorbidity. Interestingly, we observed that metabolism-related modules are deregulated in Alzheimer's disease. Moreover, in lung cancer, these modules are disrupted in an opposite way.

Conclusions: This approach will help study dynamic molecular mechanisms of diseases, especially cancers. A comprehensive reconstruction of regulated cell death signaling network will bring a better understanding of regulatory circuits and, in a near future, can be applied for therapeutic target identification and for treatment approaches optimization. This will also be useful to medical geneticists, both researchers and clinicians, who are constantly dealing with a great variety of new genes.

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P16.04D

Single-cell analysis of mutational heterogeneity in acute myeloid leukemia tumors with high-throughput droplet microfluidics

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To make possible the routine characterization of genetic diversity within cancer cell populations, we developed a novel two-step microfluidic droplet workflow that enables efficient and massively-parallel single-cell PCR-based genomic barcoding for single-cell DNA sequencing applications. We demonstrate that the two-step microfluidic approach is required for robust DNA amplification on thousands of individual cells per run with high coverage uniformity and low allelic dropout of targeted genomic loci. To apply our single-cell sequencing technology to human tumor samples, we developed a targeted panel to partially sequence 26 genes frequently mutated in acute myeloid leukemia (AML) including *TP53, DNMT3A, FLT3, NPM1, NRAS, IDH1* and *IDH2*. Using this panel, we were able to sensitively identify SNV and indel-defined clones within AML samples and assess their distribution at the time of diagnosis, remission and relapse. We also used single cell SNVs to monitor host and donor cell populations during bone marrow transplantation (BMT), which allowed us to accurately evaluate engraftment and disease relapse. Collectively, our single-cell data indicates that clonal populations inferred from VAFs obtained from bulk sequencing data may not fully resolve the heterogeneity within tumors; moreover, the single-cell nature of our approach enabled the unambiguous identification of multiple co-occurring mutations within subclones that is not possible with bulk measurements. Collectively, our results show a greater degree of heterogeneity in AML tumor samples than is commonly appreciated with traditional sequencing paradigms and demonstrate the value of single-cell analysis for AML.

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P16.05A

Phenopolis: an open platform for harmonisation of genetic and phenotypic data

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Introduction: The molecular diagnosis of rare genetic diseases requires detailed clinical phenotypes and processing of large amounts of genetic data. This motivates large-scale collaborations between clinicians, geneticists and bioinformaticians across multiple sites where patient data are pooled together to boost the chances of solving rare cases, and validating novel genes. This motivated the development of Phenopolis, a platform for harmonisation of genetic and phenotypic data.

Methods: Using Human Phenotype Ontology (HPO) encoded phenotypes, Phenopolis is able to prioritise causative genes using different sources of evidence, such as literature search and model organism phenotype ontology analysis. Additionally, Phenopolis uncovers gene-phenotype relationships within the stored patient data through variant filtering and statistical enrichment of HPO terms. The database is implemented using a graph database for scalability which allows efficient linking of HPO, genes and variants.

Results: Phenopolis is an open-source web server providing an intuitive interface to genetic and phenotypic databases. Phenopolis is also an ideal platform for studying the pleiotropy of genes. The online version available at www.phenopolis.org, includes four example patients with inherited retinal dystrophies, to illustrate our methods.

Discussion: The Phenopolis platform accelerates clinical diagnosis, gene discovery and encourages wider adoption of the HPO in the study of rare genetic diseases. We plan on extending Phenopolis to interface with the Genomics England GenePanel app to retrieve relevant genes and contribute novel disease genes.

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P16.06B

An integrative approach to correlate clinical presentation patterns in Autism Spectrum Disorder with biological processes disrupted by Copy Number Variants (CNV) in brain genes

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Autism Spectrum Disorder (ASD) is characterized by highly heterogeneous clinical phenotypes and complex genetic architecture, rendering early diagnosis and prognosis difficult. CNVs in many genes have been implicated in ASD, disrupting specific Biological Processes (BP) eventually associated with distinct clinical phenotypes. Here we sought to identify and predict clinical patterns associated with BP disrupted by specific brain gene CNVs, using a machine learning-based integrative approach. Firstly, clustering analysis of clinical records from 2446 ASD patients from the Autism Genome Project identified two consistent and highly stable clusters, differing in ASD severity, adaptive behaviour and cognitive ability. Secondly, functional enrichment analysis of rare CNVs, disrupting brain-expressed genes in these ASD subjects, identified 15 significant BPs, including nervous system development, cognition, and protein polyubiquitination. Random Forest feature importance analysis showed that all these BP contributed positively to the classification of ASD severity, as defined by the identified clusters. Finally, a Naive Bayes classifier was trained using cluster and BP information from a subset of data, comprising the 325 individuals with highest BP information content scores. A stratified five-fold cross validated model predicted the severity of ASD phenotype from BPs, with precision of 0.82 and recall of 0.39. This study thus shows that severity predictions can be attained from BP putatively disrupted by brain-gene CNVs. However, precise predictions are only achieved in subgroups with high BP information content, and specificity is generally low. A clinical application will thus require further analysis, in much larger datasets that include detailed phenotypic information. FCT-Portugal (SFRH/BD/52485/2014)

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P16.07C

BEA: a web tool for BioMark gene expression analysis

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Biomark HD system is a high-throughput technology that performs more than nine thousands of real-time quantitative PCR (qPCR) reactions simultaneously. Although numerous statistical tools are available in the public domain for the analysis of conventional qPCR experiments, this is not the case when many qPCR dataset from high-throughput experiments have to be analysed. Therefore, there is a need to provide a user-friendly software able to analyze Biomark output data.

To meet this need, we are developing BEA, Biomark Expression Analysis, a web tool designed to analyse Real Time qPCR data from Biomark HD System across multiple conditions and replicates. Analysis includes relative quantification using normalization against a reference gene ($\Delta\Delta_{CT}$ method), and appropriate statistical testing to identify differential expressed genes between tested groups, taking into account test of normality and number of groups. The web application is written in R using the Shiny platform. BEA has been tested using Bimark 96.96 dynamic array data from myelodisplastic syndrome patients.

BEA will help scientists perform gene expression analyses by interactively visualizing each step with customizable options and intuitive graphical user interface. The program provide comprehensive results visualization, downloadable plots and a summarizing report, and it would not require programming knowledge. BEA will be an open source software freely available online.

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P16.08D

Network diffusion for the integrative analysis of multiple "-omics": case studies on breast cancer

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Introduction: The generation of multiple omics from the same biological system poses challenges in data analysis and interpretation. We propose a network-based approach that prioritizes genes considering their network (functional) proximity to altered genes, according to different types of experimental evidence. We compare our multi-omics gene score with those of single omics analyses, using breast cancer (BRCA) somatic mutation and gene expression from TCGA. We also identify functionally related genes analysing somatic mutations in primary tumors (PT) and PT-derived BRCA initiating cells (BCICs).

Materials and Methods: TCGA data (49 subjects) were collected using TCGAbiolinks. Fresh-frozen PTs and non-tumoral specimens from blood were collected from 8 patients at IRCCS-INT; BCICs were isolated at early *in vitro* passages; the resulting samples (PTs, BCICs and blood) were whole-exome sequenced; mutations detected by at least two variant callers were considered. Functional interactions were collected from STRING. The network proximity of altered genes was calculated by network diffusion. BRCA genes were collected from COSMIC, OMIM, INTOGEN and DOCM.

Results: The network-based analysis increased the overlap between the significant genes associated with each omics. The proposed multi-omics gene score prioritized a higher number of BRCA genes than the analysis of each omics on its own. The score underlined potential members of pathways that suggest functional links between mutations in BCICs and PTs.

Conclusions: Network diffusion-based integrative analysis underlines links among different types of evidences, prioritizing omics-specific and common players within the human interactome.

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P16.09A

Increased BRCA1/2 classification confidence: comparison of ACMG to public database classification - call for systematic molecular profiling

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Introduction: Detection of certain BRCA1/2 mutations may be indicative of risk for breast or ovarian cancer. Confident determination of the clinical significance of BRCA1/2 variants is therefore critical to geneticists, patients, and medical socioeconomics. Lack of molecular characterization for many variants and unknown association to disease complicate genetic test reporting. One approach to interpret germline variants is provided by ACMG guidelines based on five-class clinical categorization.

Methods: We investigated the ACMG classification of a breast and ovarian cancer study with respect to criteria that affect ACMG-interpretation. We (a) compared variant-annotations from public databases, (b) assessed the contribution of expert-validated publications, and (c) calculated an independent ACMG-classification.

Results: Combined assessment of variant pathogenicity derived from public database annotations requires caution. Pairwise comparison of public database content showed up to 15 and 22% discrepancy among BRCA1/2 variant-annotations, respectively. While public database consensus was comparable to the ACMG-classification of the investigated study, incorporation of additional information from published functional studies further ascertained confidence. Incorporation of our curated content to ACMG guidelines revealed up to 84 % and 78 % less 'likely-benign' or 'likely-pathogenic' BRCA1/2 classifications, respectively.

Conclusions: Our results invite initiating prospective studies that characterize the observed confidence improvement. Overall, consolidated standards with harmonized BRCA1/2 variant-annotations are essential for adept clinical classification. While patient information is a key- parameter, one other challenge is supporting the geneticist's expertise with curated evidence. We anticipate that tools reliably combining these factors will systematically augment rational management of associated medical treatment- and cost-options by further ascertaining decision-making.

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P16.10B

Generation of the Cancer Pathway Prototype - a platform for predictive cancer pathway modeling

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CanPathPro is developing a bioinformatics concept and the associated computational tools required for the translation of highly complex and heterogeneous omics data into predictive modelling of cancer signaling. The approach is utilizing existing data and databases relevant to cancer signaling as well as CanPathPro-generated mouse omics data obtained from mouse cancer models, for practical testing and validation of the system. These data are integrated by an existing modelling computational platform, to identify signaling networks critical for breast and lung cancer development. The in silico-generated predictions are validated using (i) organotypic systems recapitulating breast and lung lesions, (ii) well-phenotyped, existing and CanPathPro-generated mouse models.

The validated bioinformatics concept will be built into the CanPathPro prototype: A tripartite tool for the (i) generation and (ii) integration of quantitative mouse omics data, followed by (iii) predictive in silico modelling of cancer signaling pathways and networks in mouse models.

CanPathPro has made good progress towards expanding and building a bioinformatics modelling platform. Well defined mouse models were amplified and omics analysis was carried out of mouse models, mouse model derived cells and organoid models for validation of the predictive modelling platform. The combined experimental- & systems biology validation platform, will be utilized in generating and testing cancer signaling hypotheses in biomedical research.

The innovative approach taken by CanPathPro is set to have broad and significant impact on diverse areas, from cancer research and personalized medicine to drug discovery and development, and ultimately improving outcomes for cancer patients.

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P16.11C
Clinical decision support system for differential diagnosis of monogenic syndromes and microdeletion and microduplication syndromes

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Introduction: The existence of many forms of rare monogenic syndromes and unbalanced chromosome aberrations with similar phenotypes create difficulties in diagnosis and require the development of tools for differential diagnosis before the appointment of genetic testing to confirm the final diagnosis.

Materials and Methods: Available public INTERNET sources were used to fill the knowledge base of the decision support system. For the formation of the catalogs of monogenic forms the information from OMIM, ORPHANET was analyzed. Description of the clinical picture according to the literature data was carried out on the basis of modified and extended ontology HPO. The catalog of chromosome aberrations was formed on the basis of dbVar, OMIM and ISCA. The program was implemented on the basis of the platform xGEN IDS and xGenCloud service.

Results: We developed a clinical decision support system (CDSS), including 10934 monogenic syndromes and 17713 aberrations with clinical picture. For each symptom described the clinical picture of the syndrome, the presence information indicates the frequency of occurrence among the patients in five categories (obligatory, very frequent, frequent, rare, very rare), type of symptom in the manifestation of symptoms (congenital, acquired), the time of occurrence of the symptom. The differential diagnostic algorithm in the dialog mode ensures the elimination of syndromes and brings the user to the final diagnosis.

Conclusion: The using of CDSS can significantly optimize the work of the doctor, simplifying differential diagnosis and, therefore, improve the effectiveness of medical and genetic counseling of patients with monogenic syndromes and chromosome aberrations.

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P16.12D
The role of cell-free mitochondrial DNA in neurodegenerative disease

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Introduction: Previous research has indicated that cell-free mitochondrial DNA (ccf-mtDNA) copy number (CN) is significantly depleted in PD and AD CSF versus controls, suggesting this may serve as a viable biomarker of broad neurodegeneration. Therefore further study, broadening the clinical spectrum are warranted. To address this we investigated the role and integrity of ccf-mtDNA in the development and progression of several neurodegenerative diseases (NDD) including; PD, DLB, AD and MS.

Methods: We have used quantitative PCR to measure ccf-mtDNA CN in ventricular and lumbar CSF samples from various NDD, to assess the viability of ccf-mtDNA as a disease biomarker. Secondly, we have investigated both mitochondrial and cell-death marker protein levels of these samples using western blotting and ELISA, correlating these to ccf-mtDNA, to determine the origin and effect of ccf-mtDNA. Finally we have assessed the integrity of ccf-mtDNA using NGS, comparing ccf-mtDNA to reference tissue samples.

Results: Our results support our hypothesis that low ccf-mtDNA is a biomarker for neurodegeneration, particularly in PD. However, we found no correlation to ccf-mtDNA and mitochondrial or neuronal protein levels, despite significantly reduced levels in PD versus controls. Furthermore, Braak stage and dementia severity did not correlate to ccf-mtDNA.

Conclusions: Low CSF ccf-mtDNA is a viable biomarker for neurodegeneration, raising the potential that this could be used as a diagnostic predictor of disease onset and progression, however more work is needed to validate this. In addition, our work sheds light on potential pathomechanisms of disease development of age-related neurodegenerative disorders.

H. Lowes: None. **M. Kurzawa-Akanbi:** None. **A. Pyle:** None. **G. Hudson:** None.

P16.13A

InCAS: an integrative annotator of human copy number variants using functional genomic features

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Introduction: Copy Number Variants (CNVs) are defined as deletions or duplications of stretches of contiguous nucleotides typically larger than 1,000 base pairs. Human CNVs are known to be involved in several diseases such as intellectual disability, autism, schizophrenia and congenital anomalies, generally due to the presence of dosage-sensitive genes. However, CNVs frequently do not target protein-coding genes, thereby hitting interspersed functional DNA regions, with clinical significance that need to be largely explored.

Materials and Methods: InCAS is an annotation tool that draws information on gene and functional DNA elements as well as of known and clinically significant CNVs from several third-party databases. Gene annotations are obtained from RefSeq and GENCODE databases. Non-coding genes (microRNAs and lnRNAs) annotations are retrieved from miRBase and lncRNAdb. Functional elements (e.g. conserved transcription factor-binding sites, microRNA regulatory sites, ultraconserved region, promoter and enhancer predictions) are annotated through different other sources of annotations. Polymorphic CNVs are retrieved from the Database of Genomic Variants (DGV). CNV-related clinically significant data are obtained from OMIM, Deciphering Developmental Disorders (DECIPHER) and ClinVar databases. The whole framework is implemented in Python and Flask and deployed to a running instance of Nginx. Data are stored in MongoDB and accessible through both a mobile-first web interface and a RESTful interface for programmatic access.

Results: InCAS allows to comprehensively annotate CNVs using continuously updated information of protein-coding and non-coding elements as well as of clinically significant CNVs. It can be advantageous for the assessment of the functional effect and clinical significance of CNVs.

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P16.14B

Comparison of clinical yield between a single NGS assay vs. combined array and NGS approach for clinical testing

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We present an approach that utilizes NGS technology to effectively and simultaneously extract CNV, AOH and SNV data using a single assay and integrate this data into a single analysis pipeline. This approach is contrasted with the more traditional way of using Chromosomal Micro-Arrays (CMAs) for CNV and AOH detection and the use of NGS technology for detection of Sequence Variants. We have demonstrated the utility of such an integrated filtering and visualization framework on a set of anonymized clinical samples referred for various constitutional conditions. These samples have been processed for molecular testing using the Illumina TruSight One panel with targeted coverage across ~ 4800 genes. The established laboratory informatics pipeline is used for sequence alignment and variant calling followed by identification of CNV and AOH regions via the BAM MultiScale Reference (MSR) algorithm. Results of CNV analysis when compared with Affymetrix 750k array data for the same samples has generated over 80% concordance with the remaining 20% being attributed to the differences in probe coverage and/or targeted sequencing read-depth. The whole genome CNV and AOH analysis has been combined with HPO directed SNV analysis and compared with the lab's existing gene-panel testing pipeline. The results of this analysis show an overall increase in clinical yield of over 10% over the traditional approach. A detailed comparison of the two approaches will be presented.

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P16.15C

Validation of an ultra-fast CNV calling tool for Next Generation Sequencing data using MLPA-verified copy number alterations

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DNA Copy Number Variations (CNVs) are often associated with human disease and, as such, important for clinical diagnostics using genetic testing. Next Generation Sequencing (NGS) has caused a massive increase in genetic testing, but accurate calling of CNVs has remained challenging. Mahamdallie et al (2017) has published high quality sequencing data from a targeted NGS assay (TruSight Cancer Panel) together with Multiplex Ligation-dependent Probe Amplification (MLPA) validated positive and negative CNV results for 96 independent samples (ICR96). This is a highly reliable resource to evaluate the accuracy of NGS CNV calling tools. We validated the GENALICE MAP CNV calling tool with this ICR96 benchmark dataset. Our results show that GENALICE MAP has high sensitivity and detected 65 out of 68 MLPA-verified CNVs. The verified CNVs occur in 66 samples and include 20 cancer predisposition genes, such as *BRCA1*, *BRCA2*, *TP53*, *PTEN* and others. We also assessed its specificity by examining 26 genes with normal copy numbers across 69 samples. Compared to other CNV calling tools, GENALICE MAP CNV calling is exceptionally fast. We observed turnaround times of less than 10 seconds per sample. Processing exome and full genome samples takes less than 10 seconds and 2 minutes respectively. In conclusion, GENALICE MAP CNV calling provides highly accurate results in almost real time.

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P16.16D
Secondary co-expression networks for the study of gene roles in multiple cell types

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Gene co-expression (CE) network analysis is an effective approach for discovering gene clusters of functional significance. Normally, CE assumes a gene has a single set of "co-relationships", and captures the most evident clustering, not necessarily the most important. With this in mind, we pursue the identification of secondary networks. We focus on secondary cell type-specific networks since CE (WGCNA-based) networks are highly driven by cell type and we know that genes vary considerably in terms of their cellular specificity. We understand gene expression as an additive model of eigengenes that we use for regression-based cell type-specific signal correction. We create a co-expression network from GTExV6 frontal cortex samples and use its eigengenes to "clean" gene expression and create a new network for each cell type. Using this approach secondary cell type associations were identified for neurons (403 genes), astrocytes (205 genes), oligodendrocytes (190 genes) and microglia (115 genes) to generate a 2.8 - 11.0% increase in the predicted cell type-specific gene sets. We assess whether these secondary gene assignments were significantly enriched for true cell type-specific associations using public transcriptomic data generated by immunopanning of human frontal cortex (http://web.stanford.edu/group/barres_lab/). We detect significant enrichments in cell type-specific gene expression for genes newly assigned to astrocyte, neuron, microglia and oligodendrocyte-specific modules, with amongst the most significant enrichments detected for the reassignment of astrocytic genes to the neuronal set (empirical p-value $<9.9 \times 10^{-5}$). Thus, we demonstrate the utility of secondary co-expression networks for identifying additional and potentially important novel gene clusters.

J.A. Sánchez: None. **R.H. Reynolds:** None. **J. Hardy:** None. **M. Ryten:** None. **J.A. Botía:** None.

P16.17A
Advances in computer-assisted syndrome recognition by the example of inborn errors of metabolism

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Significant improvements in automated image analysis have been achieved over the recent years and tools are now increasingly being used in computer-assisted syndromology. However, the ability to recognize a syndromic facial gestalt might depend on the syndrome and may also be confounded by severity of phenotype, size of available training sets, ethnicity, age, and sex. Therefore,

benchmarking and comparing the performance of deep-learned classification processes is inherently difficult.

For a systematic analysis of these influencing factors we chose the lysosomal storage diseases Mucolipidosis as well as Mucopolysaccharidosis type I and II, that are known for their wide and overlapping phenotypic spectra. For a dysmorphic comparison we used Smith-Lemli-Opitz syndrome as another inborn error of metabolism and Nicolaides-Baraitser syndrome. A classifier that was trained on these five cohorts, comprising 288 patients in total, achieved a mean accuracy of 62%.

We also developed a simulation framework to analyze the effect of potential confounders, such as cohort size, age, sex, or ethnic background on the distinguishability of phenotypes. We found that the true positive rate increases for all analyzed disorders for growing cohorts (n= [10...40]) while ethnicity and sex have no significant influence.

The dynamics of the accuracies strongly suggest that the maximum distinguishability is a phenotype-specific value, that hasn't been reached yet for any of the studied disorders. This should also be a motivation to further intensify data sharing efforts, as computer-assisted syndrome classification can still be improved by enlarging the available training sets.

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P16.18B
Efficient curation and ontology mapping of clinical and phenotypic data

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Heterogeneity within and between data sources is one of the primary impediments to assembling rich sets of phenotypic and clinical data. Such heterogeneity can arise from multiple sources: data collection practices evolve over time, standards for data encoding may be missing, or the data (e.g. EHR data) may simply originally have been collected for another purpose. Additionally, narrative text, such as medical statements, are inherently unstructured and require curation before analysis.

Irrespective of where heterogeneity derives from, curation of the data is necessary for efficient, accurate and reproducible data analysis. In addition to correcting errors and inconsistencies in the data, curation extends into enriching the data through mapping it to relevant biomedical ontologies, such as SNOMED CT and Human Phenotype Ontology. Efficient ontology encoding of data, in turn, is key to e.g. recently developed methods for using hierarchical structure between phenotypes in genetic association analyses.

To solve the most common issues in clinical and phenotypic data curation, we have developed the Accurate (TM) data curation and ontology mapping solution. We present results on preliminary benchmarking of the narrative text analytics on the MIMIC III data set to suggest good specificity and sensitivity for identification of biomedically relevant concepts. Additionally, we present results for benchmarking our ontology mapping approach for structured data, illustrating the large gains achieved in ontology mapping efficiency.

In summary, we here present an intuitive and highly efficient solution for curating clinical and phenotypic data, emphasizing enriching it through ontology mapping of both structured data and narrative text.

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P16.19C
Zenome as a new blockchain solution for storing, processing and exchanging of genetic data

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The main problem faced by researchers in the field of human genetics is the accession to the structured genomic and related data. In addition, most of the analyzed parameters are medical, and access to them is regulated by local rules. But, although the banks of genomic data are already actively developing, until there is no network with the real time feedback operation.In order to solve the tasks and significantly simplify the work with the data, a Zenome project was created.

It is a decentralized p2p network in which everyone can place their data and update information about themselves,

and those who wish to use this data can request permission. The technical basis of the network is the DHT Kademlia decentralized data storage protocol, and the protocol of blocking is used to fix the interaction. Also, the network will use a token-based incentive system in which researchers will pay for access to the data, while the user who provided the data will receive funds for their provision. To defeat user's privacy, a self-developed algorithm for storing open statements signed with identifiers is used. In such a network, everyone can get information about the presence of participants with a particular mutation or with one or another response, but only the owner knows the connection between the statements. Our service will make the costs for GWAS and family genetic tests significantly lower.

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P16.20D

Targeted single nucleotide editing allows correction of hundreds of pathogenic variants in hereditary diseases

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Introduction: Recently new CRISPR/Cas9 tools have been developed. Briefly nucleotide deaminases (ND) fused to dCas9 allow targeted direct conversion from C(G) to T(A) and from T(A) to C(G) without double-stranded brakes. It means that in total four types of mutations in hereditary diseases can be corrected: T>C, A>G, C>T, G>A. However ND are active in a window of several nucleotides which limits their potential usage.

Methods: ClinVar database was used to extract all known pathogenic mutations. Nucleotide surrounding of each mutation was analyzed for the available PAM sequence and for the absence of other nucleotides which can be nonspecifically changed by ND.

Results: We performed analysis of all variants registered in ClinVar and found that 54957 T(A)>C(G) substitutions constitute 18.2% of all records and 112740 C(G)>T(A) other 37.4%. 7084 (12.9%) and 18702 (16.6%) of them respectively were classified as pathogenic and likely pathogenic variants. For 3744 T(A)>C(G) and 5550 C(G) >T(A) mutations PAM sequence for Cas9 (NGG) was found in 11-23 nucleotides up or downstream which enables their conversion by ND fused to dCas9. Since deaminase is active in the window of 12 nucleotides for T>C and 6 nucleotides for C>T mutations it's important to select only those mutations which can be edited without conversion of nearby nucleotides. In total 220 T(A)>C(G) and 2002 C(G) >T(A) mutations can be targeted by dCas9-deaminase for single nucleotide editing.

Conclusion: The list of 2222 ClinVar mutations provides a valuable tool for the search of perspective targets for targeted direct DNA editing with dCas9-deaminase tools.

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P16.21A

A bioinformatics framework to identify cell subpopulations from bulk gene expression data of cancer samples

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The expression levels of biological samples are affected by the intrinsically heterogeneous cell and tissue composition. Nevertheless, when analyzing transcriptional profiles, each sample is generally evaluated in bulk without considering the presence of multiple subpopulations. This limitation might be extremely critical when analyzing gene expression profiles from cancer samples, where dissecting the mixed cell population could shed light on the intratumoral heterogeneity and on the molecular mechanisms shaping different cancer behaviors.

We built on Cibersort, a recently published deconvolution algorithm (*Newman et al., 2015*), to design a framework for the identification of cellular subpopulations from bulk gene expression of cancer samples. After testing the efficacy of the approach on mouse gene expression data, we applied the framework to a set of clinically-defined Triple Negative Breast Cancer (TNBC). Specifically, we defined a novel gene signature starting from 55 samples characterized as Luminal A, Luminal B, Her2, and TNBC subtypes by IHC. Afterward, we applied the gene signature to quantify the fractions of each subtype in a set of 357 TNBC samples. Results confirmed that 71.4% of samples was indeed enriched in the TNBC-like cell subpopulation, but also evidenced that the remaining 28.6% of samples were not TNBC-like. We are currently evaluating the correlation between the detected subpopulation heterogeneity and the clinical outcome reported for this cohort of TNBCs.

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P16.22B

Identification of Disease Causal Variant using Artificial Intelligence

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Genomic sequencing is important for diagnosing rare genetic diseases or cancer. Typically, a patient carries hundreds of thousands of genetic variants in the protein coding regions in the genome. Since variant classification is the basis for clinical diagnosis, it is often the bottle neck for patients' genomic data analysis, and for clinical reporting. In order to speed up the variant classification process, we set out to see if it is possible to use artificial intelligence/ machine learning method to find disease causal variants with satisfactory precision. Three sets of variants are identified with known labels, namely pathogenic, unknown significance and benign, respectively. Feature engineering techniques are used to figure out the best feature set to feed into the model. Total eleven selected type of variant feature families including 200 features are fed into the model for training. Proprietary machine learning algorithm based on random forest is used to learn a multi-label classification model. The learned model achieves 72.0% accuray on the held-out 20% dataset (48,211 variants) across all three labels. Particularly for pathogenic variants, we achieve 74.9% precision and 64.3% recall (F-measure 69.2%). The features that can dramatically increase F-measure for pathogenic variant classification will be presented. In conclusion, machine learning method can take into account of tens or hundreds of variant features for the variant classification process, which is labor intensive if it is done manually. A well-trained model will speed up the causal variant identification and clinical diagnosis. The lessons learned and the future direction will be discussed.

W. Li: None.

P16.23C

Trusted Friend Computing: Securely share OMICs data

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Introduction: Sharing OMICs data is becoming increasingly essential when analysing patient data. Yet many legal and ethical constraints slow down or make it impossible to share the data. This leads to a situation where many laboratories store their valuable patient data only in internal databases, unable to share them even with even others in the same consortium. In an effort to solve this problem, we developed TFC (Trusted Friend Computing), an extension to the POP-Java programming language, enabling developers to create applications that can share data and computing power in a secure way. We integrated this technology into GensearchNGS, an existing NGS data analysis software developed by Phenosystems SA, to enable users to share variant data in an easy and secure way.

Methods: We extended the open source POP-Java language to include the features needed to implement TFC. Those include the ability to create a friend network (in which computing resources can be shared) as well as the integration of encrypted communications. We also modified GensearchNGS to make use of those features to let laboratories share both summary variant statistics (e.g is a variant known by a certain laboratory) as well share computing power through distributed sequence alignment.

Results: We produced a version of GensearchNGS which lets users share their data and computing power with a selected list of friends. The underlying technology, POP-Java, has been released as open-source software. Grants: CTI no. 18781.1 PFES-ES

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P16.24D

Identification and characterization of 3′UTR mutations affecting *NFKBIZ* in non-Hodgkin lymphoma

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is an aggressive cancer originating from mature B-cells. Most somatic driver mutations characterized to date affect the coding sequence of proteins or induce loss of function or deregulated expression through deletion, translocation or amplification. DLBCL has two molecular subgroups with distinct genetic features and prognosis. A relative lack of drivers unique to the activated B-cell (ABC) subgroup, which suggests incomplete understanding of its molecular aetiology.

Materials and Methods: Using a combination of data modalities including RNA-seq, copy number arrays, whole genome sequencing and targeted sequencing of over 700

cases, we searched for patterns of noncoding mutations that may affect gene expression or mRNA structure in DLBCL. We implemented a new algorithm to identify discrete loci enriched for somatic noncoding single nucleotide variants (SNVs) genome-wide.

Results: This analysis identified numerous genes and non-coding loci that are commonly mutated in DLBCL, with many of these attributable to aberrant somatic hypermutation. Of particular interest were recurrent mutations in the 3′UTR of *NFKBIZ*. We found these to cause elevated mRNA and protein levels in lymphoma samples. Using a combination of techniques, we demonstrate that the most commonly observed variants affect a conserved structure in the mRNA. Moreover, we show that these changes increase mRNA abundance by reducing its cleavage by the RNAse MCPIP1.

Conclusions: Our results inform on mechanisms of NF-κB pathway activation in ABC DLBCL and demonstrate the importance of noncoding mutations in contributing to oncogene over-expression in this cancer.

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P16.25A
Machine learning approach for detecting epilepsy causing proteins using protein interaction data

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Introduction: Genetic factors play a role in more than half of all epilepsies. Proteins expressed by epilepsy associated genes are known to be clustered in specific biochemical pathways. This knowledge can be applied to find similar proteins and genes that are part of these pathways.

Materials and Methods: 998 expert reviewed epilepsy genes and 998 random non-epilepsy genes expressed in fetal temporal tissue (ENCODE accession number: ENCDO064AAA) were selected. Protein interaction data expressed by the selected genes was accessed from STRING database with a minimal interaction score of 0.151. Each protein was assigned 998 features based on interaction data with 998 epilepsy related proteins. Each individual feature is a combined interaction evidence score ranging from 0 for lowest interaction evidence to 0.999 for highest. Different types of support vector machine algorithms (SVMs) built with Python 3.5 and scikit-learn library were used to classify proteins based on interaction data as either epilepsy related or not. 1665 randomly selected protein interaction data was used for training and 331 for testing.

Results: C-Support Vector Classification algorithm showed best performance on the testing set with an area under curve (AUC) of 0.88, specificity of 0.84 and sensitivity of 0.93. Similar performance was observed with Nu-Support Vector Classification (AUC 0.86, specificity 0.79, sensitivity 0.92) and Linear Support Vector Classification (AUC 0.84, specificity 0.87, sensitivity 0.79) algorithms.

Conclusions: SVMs show a good performance on epilepsy associated protein detection. Machine learning approaches can be useful in novel epilepsy causing protein discovery.

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P16.26B
Reference-free eQTL analysis reveals the importance of unannotated transcribed regions in neurological and neuropsychiatric disorders

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Accurate gene annotation is required for the interpretation of risk loci identified by genome-wide association studies (GWAS). However, the complexity of splicing in the human brain has made annotation of brain-expressed genes a non-trivial task, and this may contribute to why the majority of risk loci for neurological disorders remain poorly characterised, even when relevant eQTL data exist. To address this issue, we used eQTL analysis in a reference-free manner to improve GWAS interpretation. We used the R package derfinder to identify and quantify transcribed regions from RNA-sequencing data generated from human non-diseased post-mortem brain tissue (hippocampus, putamen and substantia nigra). After stringent quality control, cis-eQTL analysis was conducted on ~460,000 transcribed regions. Strikingly, we identified a 2.1-fold increase in eQTL detection as compared to an equivalent analysis

performed on annotated regions of the genome alone. Using our own R package annotatER, which leverages reads spanning exon-exon junctions, we found that a significant proportion of unannotated transcribed regions were spliced to known genes. Combining eQTLs with GWAS hits from the NHGRI-EBI catalogue demonstrated a significant enrichment of risk SNPs for neurological disorders amongst eQTLs tagging unannotated transcribed regions. We further characterised these findings with eQTL-GWAS co-localisation analyses, and identified several eQTLs tagging unannotated regions and co-localising with GWAS hits for neurological and neuropsychiatric disorders. Our findings confirm the utility of performing eQTL analyses in a reference-free manner and demonstrate that unexplored regions of the genome can be crucial for the understanding of complex diseases.

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P16.27C

Frailomic Project: Genetics in frailty

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Introduction: The forecasted increase in the number of older people will be accompanied by an increase of those with disabilities. Disability is preceded by frailty, a disorder that encompasses changes associated with ageing, life style.

Objective: Characterize clinical and omics-based laboratory biomarkers related with frailty to diagnosis, prognosis and monitoring of frailty.

Material and Methods: A two-phase study was designed: Exploratory and Validation phase in a well-established prospective cohort of elderly people. 256 SNPs and 20 candidates genes were selected taking into account published literature related with frailty. Selected SNPs were genotyped in a total of 1515 samples (TOLEDO and AMI cohorts) and expression analysis of candidate genes was performed in 819 samples (TOLEDO cohort). Results were harmonized using a minimal model to identify and select significant biomarkers.

Results: From the exploratory phase, 4 SNPs (rs17286758, rs2067051, rs6657616, rs2568958) and 5 candidate genes (*NFE2L2, TP53, TXNRD1, HMOX2, HIF1A)* statistically significant associated to frailty were selected to be tested in the validation phase in a second independent series formed by a total of 1000 samples from ENRICA, EXERNET, TOLEDO cohorts for genotyping and 700 samples from EXERNET and MAPT Cohorts from expression analysis. The results are currently being analysed.

Conclusion: The results obtained in this set of genetic testing would enable the performance of a close following-up of these patients, or even prevent possible health-related problems conditions, thus increasing its quality of life, while reducing direct and indirect derived cost of the national health care system.

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P16.28D

Bioinformatic pipeline validation for the application of next generation sequencing in genetic testing

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OSR Hospital introduced NGS for genetic testing but accurate identification of genomic variants is a critical factor which may affect sensitivity and specificity.We present the validation of a bioinformatic pipeline to detect germline variants in inherited diseases. The pipeline was first applied to 66 patients with known genotype sequenced with 4 different Illumina panels using Illumina platforms. We focused our attention to genes specific for the patient disease considering a total of 9 different disorders. The pipeline performs all the steps necessary to generate a diagnostic report:

alignment, variant calling, filtering for high quality variants and high resolution evaluation of coverage statistics. The pipeline is able to detect very small regions (<20 bp) with mean coverage <20X. We tested different variant callers and we highlighted differences in called variants. In the end we decided to include 3 different callers in our pipeline: Freebayes, GATK HaplotypeCaller and Illumina commercial software. We obtained a specificity ≥ 94% and a sensitivity ≥99% with differences between variant caller and sequencing panels. In particular Illumina pipeline performs better in SNV detection while HaplotypeCaller and Freebayes in INDELs (up to 40 bp). Combining the results of these three softwares we were able to detect all the 427 known variants in our dataset. Moreover, 4 samples were previously sequenced with a different NGS method and we had a high confidence SNV dataset with 100% concordance with previous results.Overall our pipeline allows high confidence in variant detection with a low probability of mutations loss.

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P16.29A

CYP21A2 mutation update: Comprehensive analysis of databases and published genetic variants

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Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroidogenesis. Disorders in steroid 21-hydroxylation account for over 95% of patients with CAH. Clinically, the 21-hydroxylase deficiency has been classified in a broad spectrum of clinical forms, ranging from severe or classical, to mild late onset or non-classical. Known allelic variants in the disease causing CYP21A2 gene are spread among different sources. Until recently, most variants reported have been identified in the clinical setting, which presumably bias described variants to pathogenic ones, as those found in the CYPAlleles database. Nevertheless, a large number of variants are being described in massive genome projects, many of which are found in dbSNP, but lack functional implications and/or their phenotypic effect. In this work, we gathered a total of 1,340 GVs in the CYP21A2 gene, from which 899 variants were unique and 230 have an effect on human health, and compiled all this information in an integrated database. We also connected CYP21A2 sequence information to phenotypic effects for all available mutations, including double mutants in cis. Data compiled in the present work could help physicians in the genetic counseling of families affected with 21-hydroxylase deficiency.

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P16.30B

Genomic Feature Prediction Models

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We have developed predictive models that use prior biological information on genomic features that more accurately predict genetic predisposition. Genomic features, consisting of a set of genetic markers, are regions of the genome that are linked to some external information (e.g. prior QTL information, genes, biological pathways, gene ontologies, sequence annotation). The main idea underlying this modeling approach is that these regions are enriched for causal variants affecting a specific trait. This is accounted for in our model by using prior information on the relative contribution of different genome regions to the overall trait heritability. This information is used for differential shrinkage of single marker effects allowing markers to contribute differently to the overall genetic predisposition which in turn leads to more accurate predictions. The predictions are based on a genomic feature best linear unbiased prediction (GFBLUP) model implemented using a Gauss Seidel (GS) method. In order to handle very large genotype-phenotype data sets (e.g. UK Biobank) a matrix-free GS method based on adjustment of residuals was used for solving the linear equation systems. Our simulation studies show that it is possible to further increase the accuracy of genomic prediction for complex traits using this model, provided the genomic features are enriched for causal variants. Our GFBLUP model using prior information on genomic features enriched for causal variants can increase the accuracy of genomic predictions in populations of unrelated individuals and provides a formal statistical framework for leveraging and evaluating information across

multiple experimental studies to provide more accurate predictions.

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P16.31C

Predictive model for metastasis and recurrence in head and neck cancer using amplified genes of tumor samples

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Introduction: There is an urgent need to develop effective diagnostic and prognostic approaches for head and neck squamous cell carcinoma (HNSCC). This study aimed to implement a model of prediction of recurrence and metastasis using a genome-wide characterization of HNSCC samples.

Material and Methods: The genomic characterization of tumor samples in a 102 HNSCC patients' cohort was performed using array comparative genomic hybridization technique, Agilent 4x180K. We identified the most statistically significant signalling pathways for amplification and deletion and the respective altered genes, using the *Homo.sapiens* package in R and the overrepresented pathways were determined, using the *limma* package. A genomic signature that distinguishes between patients with metastasis/recurrence from those without, was determined using the LASSO regression analysis. The selected genes were then used to calculate the model's performance and prediction accuracy in a logistic regression classifier with balanced training and test sets. This genomic signature was validated in an independent cohort of 176 patients from the TCGA database.

Results: The developed predictive model using 17 amplified genes showed a good accuracy (78%, CI95% [54; 88] %) and was validated in an independent population (TCGA) (66%, CI95% [50; 80.3] %). This genomic predictive model comprises regions from chromosomes 4, 5, 7, 8, 10, 11, 16, 17, 18 and 22, where are mapped several members of signaling pathways related to carcinogenesis process.

Conclusion: This genomic predictive model for recurrence and metastasis development may help in a more practical and individualized patient management and, eventually contribute to a more targeted drug design.

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P16.32D

Dante - genotyping of complex and expanded short tandem repetitions

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Introduction: Short tandem repeats (STRs) are DNA stretches in which short sequences (2-6 nucleotides) are repeated several times. Since STRs have many important biological roles and belong to the most polymorphic parts of the human genome, they became utilized in several molecular-genetic applications. Genotyping of STR alleles, therefore, was of high relevance during the last decades. Despite this, massively parallel sequencing (MPS) still lacks the analysis methods to fully utilize the information value of STRs in genome scale assays.

Materials and Methods: We propose an alignment-free algorithm for genotyping and characterizing STR alleles based on sequence reads originating from STR loci of interest. The method accounts for natural deviations from the expected sequence, such as variation in the repeat count, sequencing errors, ambiguous bases, and complex loci containing several different motifs. We implemented a correction for copy number defects caused by the polymerase induced stutter effect and prediction of STR expansions that, according to the conventional view, cannot be fully captured by inherently short MPS reads.

Results: We tested Dante on simulated data sets and on data sets obtained by targeted sequencing of protein coding parts of thousands of selected clinically relevant genes. In

both these data sets, Dante outperformed HipSTR and GATK genotyping tools. Furthermore, Dante was able to predict allele expansions in all tested clinical cases.

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P16.33A
MiBioGen: a new consortium for meta-analysis of human genome-microbiome association

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Increasing evidence that the gut microbiome plays a role in human health calls for further investigations into the factors that determine the structure of the symbiotic relation between host and microbes. As demonstrated by the initial genome-wide association studies on microbiome composition published in recent years, the host genome is clearly one of these factors. Even so, high inter-individual variability in the gut microbiome and its capacity to respond to environmental exposures requires large, multi-ethnic, population-based study designs to ensure sufficient statistical power for detecting genotype-microbe associations. To this end, we have established a collaborative initiative, MiBioGen, that joins 18 population-based cohorts comprising more than 20,000 samples for which both genetic (genome-wide genotyping platforms) and microbiome (16S rRNA gene taxonomic profiling) are available. We developed a standardized pipeline to harmonize the different platforms and methods used to generate the microbiome data, such as the method of faecal DNA purification and the selected 16S gene variable region, and confirmed that this pipeline successfully reduced the technical biases between the cohorts. A preliminary analysis on ten most abundant bacterial genera successfully replicated well-established microbe-SNP associations, such as the functional LCT-Bifidobacterium linkage, and identified five novel genome-wide associations. To our knowledge, this is the largest consortium devoted to microbiome GWAS, and we aim to bring new knowledge to the rapidly expanding field of microbiome research.

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P16.34B
Expanding the scope of the GWAS Catalog to improve drug target identification and prioritisation

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The identification of disease-associated genetic loci and validation of drug targets are overarching drivers of scientific research. Open Targets provides robust integration of evidence from different public data sources to associate genes, proteins and pathways with diseases, with the aim of prioritising targets for drug discovery. A major source of genetic evidence is the NHGRI-EBI GWAS Catalog, a manually curated open resource repository of all published GWAS results. As of January 2018, the GWAS Catalog contains 4,847 studies and 56,935 unique SNP-trait associations from 3,280 publications.

To date, curation has been limited to array-based GWAS including association analysis of >100,000 SNPs with genome-wide coverage and SNP-trait associations with a *p*-value $<1x10^{-5}$. Together with Open Targets, we are working on expanding the scope of the Catalog to:

1) include association studies carried out using targeted arrays (such as MetaboChip, ImmunoChip and exome arrays) to increase the number of causal variants that focus on immunologic, metabolic and oncologic phenotypes (key therapeutic areas for Open Targets). Curation of fifty-five

targeted array publications for inclusion in the GWAS Catalog is ongoing.

2) develop a comprehensive database of all available GWAS summary statistics (with no *p*-value cut-off) harmonised across studies to enable easy comparison and downstream analysis. These data will be made publicly available via the GWAS Catalog website and, in the future, through an application programming interface (API).

This collaboration aims to vastly increase the number of available disease associations, ultimately leading to improved target identification and disease prognosis.

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P16.37A

Construction of a multiethnic HLA-imputation reference panel

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Genotype imputation of the Human Leukocyte Antigen region (HLA), has become increasingly popular in the last years. In the research setting, imputation helps avoid costs for wetlab-based HLA typing and thus renders large-cohort analyses of the HLA feasible. Here, we present a high-quality multiethnic reference dataset based on genotypes measured with the Illumina Immunochip genotyping array including samples from Europe, China, India, Iran, Japan, Korea and Malta and an admixed African American (AA) dataset. We typed more than 1,300 samples from the aforementioned eight different ethnic backgrounds using an in-house high-resolution NGS-based approach and our allele-calling tool HLAssign (Wittig et al., 2015). Manually curated 4 digit-level alleles for the classical class I and II loci were included in the reference panel. Using extensive cross validation and different combinations of reference datasets, we analysed imputation outputs of HIBAG (Zheng et al. 2014) and Beagle (Browning et al., 2016). While HIBAG had a mean imputation accuracy across all loci of 0.96 ±0.03 for the AA, 0.99 ±0.02 for Malta and Europe and 0.98 ±0.2 to 0.4 for the remaining cohorts, Beagle had an accuracy 0.97 ±0.02 for the AA, 0.99 ± 0.02 and 0.98 ±0.02 to 0.03 for the other cohorts. While median accuracies across the loci are higher for HIBAG than for Beagle, mean accuracies are slightly higher for Beagle using our in-house dataset only. In summary, both Beagle and HIBAG are able to impute our multiethnic dataset with high accuracies, making the use of this dataset for future research promising.

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P16.38B

On the length, weight and GC content of the human genome

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Introduction: Basic parameters commonly used to describe genomes including length, weight and relative guanine-cytosine (GC) content are widely cited in absence of a primary source.

Materials and Methods: By using updated data and original software we determined these values to the best of our knowledge as standard reference for the whole human nuclear genome, for each chromosome and for mitochondrial DNA. We also devised a method to calculate the relative GC content in the whole messenger RNA sequence set and in transcriptomes by multiplying the GC content of each gene by its mean expression level.

Results: The male nuclear diploid genome extends for 6.27 Gigabase pairs (Gbp), is 205.00 centimeters (cm) long and weighs 6.41 picograms (pg). Female values are 6.37 Gbp, 208.23 cm, 6.51 pg. The individual variability and the implication for the DNA informational density in terms of bits/volume were discussed. The genomic GC content is 40.9%. Following analysis in different transcriptomes and species, we showed that the greatest deviation was observed in the pathological condition analysed (trisomy 21 leukemic cells) and in *Caenorhabditis elegans*.

Conclusions: Our results may represent a solid basis for further investigations on human structural and functional genomics while also providing a framework for other genome comparative analysis.

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P16.39C

Investigating the genetic architecture of hypertension through combined analysis of genome-wide association studies (GWAS) data and the human protein interaction network

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Introduction: Hypertension is a major cardiovascular disease and stroke risk factor. To-date, many genes have been suggested as associated to blood pressure regulation through polycentric GWAS. These findings can be validated and extended, if analyzed in the context of the protein interaction network where proteins encoded by disease-associated genes may form functional subnetworks. Our objective is to combine hypertension GWAS with relevant protein interactome data for the elucidation of blood pressure regulation mechanisms and prioritization of hypertension predisposing genes.

Materials and Methods: Primary hypertension GWAS data were retrieved from recent studies referring collectively to approximately 1,200,000 samples. The hypertension-related protein interactome was reconstructed using the PICKLE meta-database (www.pickle.gr) and analyzed using the Cytoscape software. Gene clustering and functional annotation was performed using the DAVID platform.

Results: About 350 hypertension-associated genes were retrieved from GWAS. The hypertension-associated protein interactome analysis revealed a subnetwork of 81 interconnected proteins; some, e.g. NPR1, GJA1, MME, are known anti-hypertension drug targets. Topological & functional analysis of this subnetwork revealed four pathways connected through a core of 10 proteins including NOS3 and CRK. These pathways contribute to the: (i) regulation of myocardial contraction, (ii) blood circulation, (iii) cardiac tissue morphogenesis and (iv) pressure-natriuresis mechanism.

Conclusions: This study furthers our understanding on the molecular architecture of hypertension, elucidates disease modules with a central role in abnormal blood pressure regulation and prioritizes hypertension predisposing genes. Supported by: Hellenic Foundation for Research and Innovation (HFRI) PhD scholarship grant; NSRF 2014-2020 ELIXIR-GR (MIS 5002780) and BITAD (MIS 5002469) projects.

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P16.40D

Exploring molecular interactions by clustering analysis of similarity scores from next-generation phenotyping approaches

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Introduction: Recent advances in computer vision and machine learning resulted in the next-generation phenotyping (NGP) tools for syndromology. Deep convolutional neural networks such as DeepGestalt in Face2Gene have been trained on thousands of patient photos with confirmed molecular diagnoses and learned phenotype representations of multiple disorders. These systems of artificial intelligence support clinicians in the diagnostic workup of patients and even excel human experts in certain classification tasks. We therefore wondered whether this unprecedented sensitivity to detect mutation-specific patterns in the facial gestalt could also be used to reveal information about gene function.

Materials and Methods: We designed the knowledge base Deep Phenotyping for Deep Learning (DPDL) as a public resource to compile similarity scores from NGP tools and performed a cluster analysis on currently 1216 cases with monogenic disorders.

Results: A prominent cluster was formed by BRAF, PTPN11, NRAS and other genes of the MAPKinase pathway that result in phenotypes such as Noonan, LEOPARD or cardiofaciocutaneous syndrome and which are considered similar. Likewise, many genes linked to inborn errors of metabolism also clustered, mirroring a higher-level feature that is referred to by clinicians as 'coarse facies'. Furthermore, we also found genes involved in chromatin remodeling to be near neighbors, even if no superordinate joint feature exists in medical terminology to describe the associated diseases.

Conclusion: We were able to reproduce molecular interactions by information encoded in the facial gestalt by using NGP tools. Thus, the phenotype space exploration is a promising subject in the characterization of gene function.

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P16.41A

reduSNP: used for linkage disequilibrium-pruning

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As cohort sizes keep increasing (>500,000), it has become vital to avoid data redundancy, data duplication, reduce the influence chromosomal artifacts, and facilitate replication. Linkage disequilibrium (LD) pruning is used to decrease an initial variant list to a few representatives and can also be leveraged to find variants that were not genotyped.A limited number of software tools perform LD-pruning, however, of those available we were unable to find a tool that did not require one or more of the following steps: [i] prior ranking of SNPs according to most significant p-value; [ii] need to provide the actual genotype dataset or reference panel; [iii] perform multiple LD-pruning analysis with different correlation coefficient values; [iv] require either chromosome number and/or position and if absent, then have to create a dummy variable; and [v] must require variants to be on the same chromosome.

We introduce reduSNP a lightweight, stand-alone, freely available command line tool that responds to each of the aforementioned "shortcomings" with the highlight on minimum to no pre-processing of input information.

It prunes SNPs and short (insertions/deletions) InDels based on the European ancestry 1000Genomes Project Phase3 LD values. To execute reduSNP through a series of automated steps a minimum of two parameters are required namely a list of non-ordered variants; and (multiple) normalized coefficient of LD (D') or correlation coefficients (r2). All output results are then comprehensively logged featuring suggested reasons to why variants could not be mapped.

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P16.42B

Functional effects from loss of chromosome Y (LOY) in single cells estimated by genome-wide transcriptional analyses using the 10X Chromium platform

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Men live shorter lives than women however the factors contributing to this difference remains elusive. Recent research indicates that the loss of the Y chromosome (LOY) in blood can explain parts of this difference, as it have been associated both to cancer mortality and Alzheimer's disease.

In order to investigate the effect of losing the Y chromosome we have performed single cell sequencing of 12 elderly males using the 10X genomics chromium system. Using the transcriptomes of each sequenced single cell we are able to use clustering techniques together with t-Distributed Stochastic Neighbor Embedding (tSNE) to determine the fraction of LOY cells in each major PBMC type. We can show that the results from this analysis compares well with the results from the Illumina Infinium QC Array-24 kit on FACS sorted cell fractions from the same individuals.

We have so far sequenced approximately 37 000 cells and have assigned cell-type and LOY status to each of these. This allows us to use the information from a large amount of sequenced single cells to find genes which have altered gene expression in LOY cells in specific PBMC populations.

The work presented here shows that single cell RNA sequencing can be used as a viable technique to identify the fraction of LOY in the PBMC populations of an individual. Furthermore it gives insight into the genes and pathways affected when losing the Y chromosome in blood.

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P16.43C

Gene interaction discovery in myelodysplastic syndromes

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The characterization of genetic interactions underlying myelodysplastic syndrome (MDS) pathology is the key to understand its molecular machinery and, as consequence, to design viable treatments. Many studies have been carried on in this direction, including MDS expression profiling and analysisof somatic mutations affecting MDS stems cells. In this study we underpin MDS-specific gene-gene interactions, i.e., gene interactions characterizing MDS cells, either because they are absent is healthy cells, or because the gene coexpression is significantly altered in MDS. We applied a matrix factorization-based, multi-omic, data-agnostic approach to predict MDS-related gene-gene interactions, integrating MDS somatic mutation data from TCGA, KEGG pathways, BioGRID gene-gene interactions, DisGeNET gene-disease relations, and Disease Ontology disease-disease similarity. Our method unveiled 320 MDS-specific gene-gene interactions as output. To validate these results, we (a) assessed if our predicted gene-gene interactions were confirmed at the protein level by using the STRING repository, and (b) verified if the predicted interactions were reflected in gene co-expression on external MDS cohorts. We found 173 (54%) of our predictions confirmed as protein-protein interactions in STRING. Even more interestingly, more than half of them (98/173) have a combined score greater than 0.7, which is the level indicated by the STRING curators as high confidence. Next, by combining four MDS cohorts from Gene Expression Omnibus data, we calculated the paired co-expression distribution of the predicted interactions in cases and controls. A Wilcoxon signed rank test found a significant difference (p-value $4.1*10^{-18}$).

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P16.44D

An automatic implementation of ACMG/AMP variant interpretation guidelines

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Introduction: Variant pathogenicity assessment in the diagnosis of genetic diseases is a complex process. A standard method for germline variant interpretation has been proposed by the American College of Medical Genetics and Genomics (ACMG) with the Association for Molecular Pathology (AMP) (PMID 25741868). Actual implementation of such guidelines in clinical routine requires the development of automated systems solving both complexity and reproducibility issues.

Materials and Methods: We have developed eVAI (enGenome VAriant Interpreter), an automatic variant interpretation system based on ACMG/AMP guidelines. Seventeen out of twenty-eight ACMG/AMP criteria are implemented integrating data from different omic-resources. We tested eVAI performance on CLINVITAE, a collection of clinically-observed benign and pathogenic variants related to a broad set of disorders. We also validated our system on 60 MYH7-related variants from ClinGen's Inherited Cardiomyopathy Panel (PMID 29300372).

Results: In CLINVITAE, eVAI achieved a concordance of 76.2% (4310/5651) on pathogenic variants and of 88.5% (7499/8462) on benign variants. On the same data we compared eVAI to InterVar, another similar tool, showing a reduction of VUS of about 45.3%. eVAI concordance was about 85.38% (289/299) and of 96.66% (168/200) on pathogenic and benign cardiovascular-related variants in CLINVITAE. Compared to CardioClassifier, a tool designed specifically for variants interpretation in cardiovascular diseases, eVAI showed a VUS reduction of 64%. Finally, 44/60 MYH7 validated variants were correctly interpreted.

Conclusions: Complexity of standard guidelines and the ever-growing number of variants detected from sequencing-based analysis hampers manual pathogenicity assessment. eVAI represents an automated support in guidelines-based variant interpretation in clinical practice.

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P16.45A

Airways microbial metagenomes of individuals with immune deficiency, asthma, cystic fibrosis or COPD

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Airway infections cause exacerbations of the underlying disease in patients with immune deficiency (ID), asthma or COPD and determine course and prognosis in most individuals with cystic fibrosis. To identify the composition of the upper and lower airway metagenomes in these patient groups during clinically stable condition, nasal lavage, throat swabs and sputa were collected from 142 CF, 35 asthma, 11 COPD and 10 ID patients. Genomic DNA was extracted and sequenced on SOLiD and Illumina platforms. In an automated pipeline, raw data were filtered, mapped onto a microbial pangenome (1,892 bacteria, 4,193 fungi/moulds and 1,153 DNA viruses) and normalized (GC-bias, genome size, human DNA). The data were analyzed for phylogeny, taxonomic classification, diversity and correlations. Disease-associated bacterial metagenomes were seen in patients with CF or ID, but not in patients with asthma or COPD. The prevalent phyla in human airways are Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria which make up more than 90% of the metagenome. COPD patients exhibited an airway metagenome dominated by *Streptococci, Rothia* and *Prevotella* similar to healthy smokers and non-smokers. Pre-school wheezers were mainly carrying a normal, but low-diversity microbial flora with *Rothia mucilaginosa* as prime and *Actinomyces* as diagnostic organisms. Besides numerous aerobes and anaerobes, patients with ID were harbouring *Moraxella* and *Haemophilus* as major pathogens CF patients exhibited a disease-specific flora, primarily Firmicutes (*Staphylococcus spp*.), alpha- and gamma- Proteobacteria *(P. aeruginosa, H. influenzae*). PCA uncovered clusters of species associated with severity and chronicity of CF lung disease.

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P16.46B

The Mouse Genome Informatics (MGI) resource: new features facilitating accessibility and presentation of mouse models of human disease

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Mouse Genome Informatics (MGI) is the premier community knowledgebase for the laboratory mouse. MGI integrates mouse genotype-phenotype datasets from biomedical publications and large-scale projects with genomic, mutation, expression, functional and human disease model data to accelerate correlative discoveries and inform genetic disease etiology and therapeutics. New MGI functionality reinforces the semantic acquisition, annotation, integration and display of phenotype and disease model information, and facilitates comparison of disease-related data across all model organism databases of the Alliance of Genome Resources (AGR). Recent enhancements include a new MGI Human Phenotype Ontology browser, incorporation of human disease-phenotype relationships from Orphanet, and new mouse embryonic lethal phenotype data from the DMDD consortium. Importantly, MGI has adopted the Disease Ontology (DO), a hierarchical classification of standardized disease concepts with cross-references to OMIM, MeSH, SNOMED and other clinical terminologies. MGI disease model annotations have been translated from OMIM terms to DO terms to optimize searches, and grouping and display of disease classes in relevant detail pages. The MGI Quick Search tool, Human-Mouse Disease Connection (HMDC), and advanced query forms now support searches by DO terms or IDs. Furthermore, the HMDC grid groups phenotype and disease data by top-level terms to enhance visualization of these data. Finally, an improved DO Browser allows users to traverse the ontology tree or graphical view, examine a selected term's definition, IDs and hierarchical relationships, identify all genes and mouse models annotated to that disease or any of its subtypes, and access the unified AGR disease report. Supported by NIH grant HG000330.

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P16.47C

The IMPC: mouse high-throughput phenotyping contributes to understanding the role of genes in human disease

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Introduction: The International Mouse Phenotyping Consortium (IMPC) is a world-wide initiative that has so far characterized over 4,000 knockout mouse lines, many for poorly understood genes (*the ignorome*), to better understand mammalian gene function and human disease.

Materials and Methods: Dedicated phenotyping, statistical and bioinformatic pipelines are collecting, analyzing and displaying data from more than 250 phenotypic parameters of embryos, young adult and aged mice.

Results and Conclusions: We will present how the latest data are contributing to previously observed biological insights and discovering new ones. First, we continue to observe a wide-ranging prevalence of sexual dimorphism, highlighting the importance of including both males and females in biomedical research. An embryonic phenotyping pipeline continues finding that approximately one-third of mammalian genes are essential for life, and these are highly correlated with human disease genes. Further, the overlap of adult mouse phenotypes with human clinical features has identified over 400 new disease models. Specialized studies have led to the identification of 52 genes associated to deafness for the first time, and a separate study found 974 genes associated to the glucose and lipid metabolism, including 23 genes associated with human diabetes via GWAS. These models fill the gap between GWAS and functional validation of complex traits, and shared regulatory features may potentially allow functional predictions for other metabolic genes via co-regulation networks. In addition, over 1500 publications indicate that IMPC mouse models or data are being widely used by the research community, for instance in cholesterol, bone formation, and halitosis research.

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P16.48D

Targeted analysis of whole genome sequence data to identify the link between sensory-immune system and Neuroticism

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Introduction: Neuroticism is a personality trait characterized by a tendency toward various negative emotions including anxiety, depression, and anger. Accordingly, this trait has been associated with several psychiatric disorders, and considered one of the risk factors for developing major depression and anxiety disorders. According to the theory, neurotic tendencies derive from an innate predisposition toward low-threshold, high-intensity, and long-lasting autonomic activation in response to sensory stimuli. Recently, we found that Neuroticism was associated with the olfactory receptor genes and gut microbiota in our previous study.

Materials: This research was performed using the Revised NEO Personality Inventory and the whole genome sequencing data (30X coverage) in 185 women, who have the extreme scores neuroticism ($\leq 10^{th}$ percentile and $\geq 90^{th}$ percentile). All sequenced DNA reads were mapped to the human genome reference (hg19) and SNV and indel VCFs were create by multi-sample joint aggregation using the gVCFs. Targeted analysis was focused on the olfactory receptor genes, neuroinflammation, inflammasome-, PAMP-, and DAMP-related genes.

Results: Following GATK best practices and VQSR filtering, 17,843,140 SNVs, 2,246,819 Ins and 2,662,425 Dels were retained. In allelic model and trend model, we found the associated variants in 13 olfactory receptor genes and 5 genes related with DAMP which have high and moderate impact in low and high Neuroticism groups.

Conclusions: This study provide insights for Neuroticism-linking genetic variations of sensory and immune system.

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P16.50B

MIDAS GPMS- A flexible gene panel management system

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The implementation of Next-Generation Sequencing in a clinical diagnostic setting opens vast opportunities through the ability to simultaneously sequence all genes contributing to a certain indication. However, this approach remains challenging and requires complex algorithms and significant computational resources to annotate, classify and store data. Furthermore, the need of a comprehensive system managing patient information, gene panels, samples, sequencing data and diagnostic reports becomes crucial.

To solve these issues we develop MIDAS (Multiple Integration of Data Annotation Software) a desktop based and modular constructed javafx application for data integration.

MIDAS consists of many independent modules, here we introduce the gene-panel-management-system (GPMS) module. The GPMS allows the user through a graphical user interface (GUI) to perform "CRUD-operations" (Create, Read, Update and Delete) on gene panels and their content by respecting all database constraints. Considering a gene panel may consist of many subpanels whose content

can overlap and a gene can belong to different panels, performing such operation become a non-trivial task.

Furthermore, the GPMS provides meta-information about all panels such as gene-, exon-, CDS- and base-count, department and the panel status.

Since Panel diagnostics rely on target enrichment, GPMS can also provide information about all enriched and not enriched genes of the selected panel version.

MIDAS aims to aid molecular diagnostics by simplifying and accelerating data analysis and interpretation, improving patient care. MIDAS is a part of a prospective multicentric study including clinical, diagnostic and software development partners and is funded by a grant by the Bavarian Ministry of Economy.

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P16.51C

Integration of large-scale genetic and functional datasets enable mechanistic insights into craniofacial development and disease

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GWAS for nonsyndromic cleft lip with or without cleft palate (nsCL/P) have identified 40 risk loci, mostly in non-coding regions. The functional effects of these associations remain largely unclear. Here, we applied comprehensive data integration approaches of genetic and epigenetic data sets to 1) identify potential regulatory effects of risk variants, 2) characterize potential novel variants located in functional candidate regions, and 3) to specify the manner and timing of regulatory processes affected by known variants.

We used relevant published ChIP-seq datasets from different stages of craniofacial development, i.e., human embryonic craniofacial tissue (Carnegie stages (CS) 13-17) / neural crest cells, and in-house nsCL/P GWAS summary statistics. After epigenetic imputation of histone marks, a joint bioinformatics pipeline (QC, peak calling) was applied, and chromatin segmentation procedures were performed. This provided insights into the particular involvement of different activity states at different stages and could help to differentiate which molecular processes of facial development (e.g, cell migration/fusion, or tissue formation) might be influenced by which genetic risk loci. As one preliminary example, the lead SNP at 2p21 (rs7590268) is located within an enhancer that is active in CS15/17, while being non-active in the earlier CS13/14. This correlates with a specifically strong expression of the adjacent *THADA* gene in CS15, potentially reflecting a contribution of the apoptosis-related *THADA* gene in fusion processes in facial development.

Our "functional genomics" approach will help to close the gap in the functional understanding of genetic risk loci for nsCL/P and other complex traits.

J. Welzenbach: None. **E. Mangold:** None. **M. Knapp:** None. **K. Ludwig:** None.

P16.54B

PDX Finder: An Open and Global Catalogue of Patient Tumor Derived Xenograft Models

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Introduction: Patient-derived tumor xenograft (PDX) mouse models are an important research platform to study tumor evolution, drug response and for tailoring chemotherapeutic approaches to individual patients. PDX models are produced and made available in repositories managed by academic labs, large research consortia and contract research organizations. The heterogeneous nature of PDX repositories makes finding relevant models of interest challenging.

Methods: To address this issue, The Jackson Laboratory and EMBL-EBI have co-developed the PDX Finder, a comprehensive open global catalogue of PDX models and their associated data. In support this initiative, we coordinated the community initiative to develop the PDX models Minimal Information standard (PDX-MI) that defines the information necessary for describing key elements of a PDX model including the clinical attributes of a patient's tumor, host strain, implantation and model validation methods. PDX-MI is the basis for PDX Finder's search and filtering options (e.g., tumor histology, molecular variant, drug response). Within PDX Finder, model attributes are integrated into a cohesive ontological data model that supports consistent searching across various resources. From PDX Finder, direct links to the relevant institution are provided for further collaboration. PDX Finder is collaborating with several worldwide consortia including PDXnet and EurOPDX to increase "findability" of PDX models and to advance cancer research and drug discovery.

Results/conclusion: PDX Finder is currently displaying over 1800 PDX models for a wide variety of cancers and is actively recruiting more models.The community is invited to explore and provide feedback on our portal at: www.pdxfinder.org.

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P16.55C
Who am I? The Personal Genome Project UK

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Background: The Personal Genome Project UK (PGP-UK), part of the global PGP network, creates freely available genetic, epigenetic, phenotypic, health and associated trait data from volunteer participants.

Methods: By using a multi-omics approach combined with a robust recruitment process based on open consent, we provide whole genome, methylome and transcriptome data as an open-access resource. Alongside raw data, PGP-UK provides individual variant reports for each donated genome based on multi-omic analyses. The genomic variant reports include information about ancestry, alongside genetic risk for various diseases and traits. For the first time, we have reported epigenetic variants to participants in reports including prediction of epigenetic age and smoking status based on DNA methylation markers.

Results: The PGP-UK pilot study focused on ten volunteer participants as the first collaborative citizen scientists. Alongside these, the unique genome donation framework was developed and trialed on three participants. In an additional demonstration of citizen science, a biomedical postgraduate student has become the first to analyse their own genome and epigenome as part of PGP-UK. Results from all three project areas will be presented.

Discussion: PGP-UK demonstrates that citizen science is possible, and is a key to advancing public understanding of personal genomics and health. The project has demonstrated the importance of data sharing and public collaboration in the scientific process. We conclude that the PGP-UK process and citizen science collaborative approach to genomic data creation is critical in the advancement of genomics as a tool in personalised medicine, and in public perception of personal genomics.

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P16.57A
Genome-wide association study of 1,124 protein levels in pulmonary arterial hypertension patients identifies a novel *trans*-pQTL at *ELK2AP* for Death Receptor 3

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Recent genome-wide association studies (GWAS) on blood plasma proteome in healthy individuals have identified hundreds of protein quantitative trait loci, pQTLs, both *in cis* and *in trans*. Dissecting the mechanisms of protein level variability in unhealthy individuals may help to reveal novel disease-specific pathways. Pulmonary arterial hypertension, PAH, is a rare disease leading to premature death. We conducted a GWAS using whole-genome sequencing data and 1,124 blood plasma protein levels measured with the SOMAscan platform in 128 British (discovery) and 79 French (replication) PAH patients. Each protein level was natural logarithm transformed, adjusted for age, sex and four principal components, and the resulting residuals were further inverse-normal transformed to assure normality. We discovered 21 *cis* and 6 *trans*-pQTLs at genome-wide significance corrected for multiple testing ($P < 4.45\times10^{-11}$) that replicated with nominal significance and directional consistency. These contain four novel *trans*-pQTLs at/near *ELK2AP* for death receptor 3 (DR3); *MIR4435−1* for Complement component 1 subcomponent r (C1r); *RETNLB* for Hepatocyte growth factor activator (HGFA); and *TMEM215* for Properdin. Animal studies have shown DR3, a cell surface receptor of the tumor necrosis factor receptor superfamily (TNFRSF), to emerge as a major regulator of inflammatory and autoimmune disease, and therapeutic agonists of TNFRSF25 can be used to simulate Treg expansion. *ELK2AP* is on the border of immunoglobulin heavy locus, further suggesting the role of this novel pQTL to be inflammation and immunity related. Our results provide support for previous research suggesting that inflammation and altered immune processes underlie the development of PAH.

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P16.58B
Qtlizer: Comprehensive QTL annotation of GWAS results

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Exploration of genetic variant to gene relationships can help to identify candidate causal variants and genes in Post-GWAS analyses. In this work, we introduce Qtlizer, a novel web-based tool to annotate common variants in human with associated changes in gene expression using the, to-date, most comprehensive database of published quantitative trait loci (QTL).

More precisely, we integrated 169 tissue-specific QTL datasets (one response expression QTLs, one protein abundance QTLs, all other expression QTLs) from the public domain and further included two inhouse datasets on monocytes and macrophages from the Cardiogenics Consortium.

The QTL table resulting from up to 100 inputted rsIDs (optionally, LD variants can be included) consists of columns for variant, gene, type of QTL, distance in base pairs, tissue, effect size, significance information, and origin. Additionally, we added three more attributes: (1) Co-localization: instead of defining fixed size of e.g. one mega base pair, we utilized the TAD boundaries to categorize QTLs into cis (i.e. variant and gene remain in the same TAD) and trans (2) Relevance flags and counts: these were added to each QTL to draw conclusions about its reproducibility across tissues and studies as well as its causality (3) GWAS Catalog: variants and genes are marked if listed in the catalog.

In summary, Qtlizer provides a web-based solution for annotating lists of genetic variants with QTLs from a large number of published datasets as well as from two recently released eQTL datasets. Our tool is freely available at http://www.genehopper.de/qtlizer.

M. Munz: None. **A. Schäfer:** None. **J. Erdmann:** None.

P16.59C

Use of RNA sequencing for the diagnosis of heterogenous genetic diseases

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Use of DNA high throughput sequencing (Whole-exome sequencing, WES or targeted sequencing, TS) in clinical practices have greatly improved molecular diagnosis of genetically heterogenous diseases, such as Intellectual Disability (ID) or Retinitis pigmentosa (RP). However, an important portion of the patients remains undiagnosed after the exploration of genomic coding regions. To identify additional variants not seen by WES, whole genome sequencing (WGS) is a straightforward strategy but still remains heavy and expensive. Thus, we used paired-end sequencing of messenger RNA (RNA-seq) extracted from available patients' tissues, blood and fibroblasts, to search for pathogenic variants. Eight patients with known mutations, affecting splicing or leading to the loss of expression of one allele (heterozygote deletion, nonsense mRNA decay, etc) were included, as well as ten patients with ID or RP without diagnosis after TS or WES. We performed a combined analysis of RNAseq data including 1) a variant detection comparison between RNASeq and DNAseq data using VaRank 2) the detection of splicing alterations using three different programs (rMATS, JunctionSeq and LeafCutter) 3) a differential expression study using DEseq. This 3-way analysis allowed us to retrieve the known mutations in 8/10 cases and identified interesting candidate variations in some of the other patients. A significant number of genes involved in ID and RP are expressed either in fibroblasts or blood. We therefore confirm that RNAseq from patient's skin or blood cells can be a useful approach to identify pathogenic variants in coding and noncoding regions. supported by Fondation Maladies Rares, Fondation Jérome Lejeune

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P16.60D

Seekmer: fast and accurate transcript level quantification at low sequencing depth

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Seekmer: fast and accurate transcript-level quantification at low sequencing depth

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Abstract

Achieving both fast and accurate quantification of transcript abundance in RNA-Seq experiments remains an elusive goal, with trade-offs between the high accuracy of alignment-based methods and the short runtime of alignment-free approaches for estimating the abundance of individual transcripts. We present Seekmer, a highly efficient quantification tool that leverages the relative advantages of each approach without their corresponding shortcomings. Seekmer incorporates a modified de Bruijn approach for quick sequence assignments into an alignment-free mapping, resulting in a 95% increase in speed over alignment-based tools while retaining a high accuracy as compared to both molecular and simulated data sets. We further show increased transcript discrimination at lower expression levels, thus enabling the analysis of subtle changes between different cellular systems as well as individual cells. Seekmer thus provides scalable and robust transcript quantification for current full-length bulk and single-cell RNA sequencing methods.

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P16.61A

Determination of CD3 subpopulation specific gene expression from whole blood samples and its applications

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Traditional approach to determine transcript abundance(TA) of one cell type in cell-mixture samples (e.g. Whole blood) require cell-isolation, like magnetic cell-sorting to single out target cell-types for quantification of TA. The cell-sorting procedure is labour-intense and/or expensive (e.g. single cell RNA-seq). An in-vitro diagnostics that can report TA of single cell-type without cell-sorting is needed for routine hospital laboratory operation. In this example, we used CD3 T-cell as the target leukocyte subpopulation in which transcript abundance was determined in Whole Blood samples.

A list of T-cells cell-type specific genes were defined as genes whose transcripts in the Whole Blood were predominantly contributed from T-cells (over 50%). From such T cells cell-type specific gene list, a functional reporter gene of interest was chosen as a target gene. Another cell-type specific gene with low biological variation and not affected by the disease condition was chosen as reference gene. We used real time PCR to quantify the 2 genes in Whole Blood Samples and ddCT ratio of them provided an index of the target gene expression level in T Cells.

LRRN3 was T cells cell-type specific gene. Using PRKCQ as the T cells cell-type specific reference gene, LRRN3 LS-TA was determined in Whole Blood samples which reflect the levels in isolated T Cells (R2=0.62). Furthermore, LRRN3 LS-TA measured in Whole Blood samples differentiated patients on immunosuppressant therapy from healthy controls (sensitivity >85%). Therefore, it is feasible to translate this direct LS-TA assay on Whole Blood Samples in routine hospital applications.

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P16.62B

Sparse discrete data analytics for single-cell genomics and applications to spermatogenesis

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Our study tackles three challenges facing high-dimensional sparse counts data: mathematical theory, algorithmic development, and applications. With single-cell RNA sequencing, the transcript counts contain low integers and very high rates of 0's due to shallow sampling and “drop-outs”. Since cell number (100's-1000's) is usually smaller than gene number (20,000-40,000), statistical inference tends to be under-determined; yet much information is conveyed by subsets of genes. Most current theories on statistical learning assume real-valued measurements in well-behaved distributions. We developed new theoretical support for the unique distribution properties of “double-sparse” data, with (1) low-rank structure and (2) low integers with zero-inflation. One recent effort is to adopt the Gini Index to assess if a cell devotes most of its transcripts on a small spectrum of genes, or spread them over many regulatory programs. Yet the Gini varies with the cell's sequencing depth; in fact, both the alpha and beta diversity measures exhibit dependencies with abundance and dispersion properties of the counts data. We modify classic distance measures and critically reassess existing algorithms for clustering, imputation, marker selection, and benchmark them in reusable, extendable, truth-known simulations. We apply our workflow to analyze >36K single cells from the mouse testis, finding that the transition from spermatocytes to mature sperms follow a smooth transition; four identified spermatogonia subtypes map to known differentiation processes; and nine subtypes of Sertoli cells are interspersed with spatially defined histological stages. Our experience highlights the value of iterative discovery cycles through theory-methods-applications. (Funding: 1R21HD090371-01A1, 1DP2HD091949-01, Michigan Institute for Data Science.)

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P16.63C

Evaluation of the performance of NGS bioinformatics tools for the detection of somatic mutations in autoinflammatory diseases

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Introduction: Autoinflammatory diseases represent a privileged scenario to the study of the somatic variation. First, somatic mutations are suspected to cause autoinflammatory diseases in a considerable fraction of patients. Second, DNA from blood is easily obtained. And third, separating different cell populations is also possible with standard flow cytometry procedures. However, the detection of somatic mutations from NGS presents some difficulties. Low frequency somatic mutations are at risk of being undetected with standard procedures. Also, most of the algorithms for somatic mutation analyses are optimized for cancer studies, where a tumour sample is compared with the healthy tissue. Thus, higher coverages and the modification of experimental designs and pipelines are needed to detect these variants.

Materials and Methods: We sequenced the whole exomes from blood samples of five patients with known somatic variants with a mean coverage 260X. We used somatic variant callers, VarScar2, MuTect2 and VarDict, to generate a list of possible somatic mutations. For two patients, we also separated blood cell populations in order to establish their frequencies. CNV analysis was performed using CoNIFER and XHMM software.

Results: We found 4 out of 5 variants, all but the one at lower frequency (2.8%). All others (ranging from 7.7%-31.3%) were detected by, at least, VarScan2. Estimated frequencies by experimental methods and by the NGS approach were similar in general.

Conclusion: Next generation sequencing is a tool with potential application to detect somatic mutations in autoinflammatory diseases. MDM-2014-0370-16-3 grant to M.S.-M. Grant SAF2015-68472-C2-2-R from Mineco (Spain) and FEDER to FC.

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P16.64D

Somatic Mutation detection using deep Whole Exome Sequencing on post-mortem brain tissue

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Introduction: Research into the contribution of somatic mutations to human diseases has been mostly confined to cancer genetics. Recently, studies have emerged regarding somatic mutations in neurological diseases. Most use candidate gene panel sequencing or sequencing of single cells. These methods are either limited to a subset of genes or a subset of cells. We measured all coding somatic mutations in two specific brain regions of six Semantic Dementia (SD) patients and report affected genes.

Methods: We performed whole exome sequencing on dentate gyrus (mean coverage 250x), middle temporal gyrus (500x) and blood (120x) of six SD patients. Using Mutect and GATK's Unified Genotyper, we extracted a total of 193.250 candidate mutation sites. Through a custom filtering pipeline, we retained 2.312 somatic mutations. Each mutation has a CADD score of at least 10 and is completely absent from the ExAC database.

Results: Of 2.312 somatic mutations, 736 are detected in dentate gyrus, 1.571 in middle temporal gyrus and 5 in both. Between 24 and 479 somatic mutations were found per tissue per patient. In total 2.033 genes were affected by somatic mutations. 254 genes were affected in multiple tissues or samples. The most affected gene was TTN, carrying seven unique somatic mutations in six tissues of five patients.

Conclusions: Deep WES allows measuring a wide range of coding somatic mutations within a selected tissue. Large variation exist in the number of potentially damaging mutations per patient and tissue. We identified 254 genes with mutations in multiple patients or tissues.

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P16.65A

Graph methods for validating complex variants in pedigrees and large sample cohorts

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Accurate detection and annotation of complex variants (e.g. paralogs and SVs) is a critical part of the clinical variant calling process. A challenge with SV calling is that the same event is often represented differently between samples. Nearby variants and sequence instability around the breakpoints can further complicate these issues. Thus, complex variants can be difficult to aggregate across samples leading to Mendelian conflicts, or underestimation of variant frequencies.

We have developed a joint genotyping method for complex variants based on realigning sequence reads overlapping variant locations or breakpoints to a variant graph. This method enables us to find a canonical variant representation and use it to consistently genotype a variant across many samples. Once a set of variants is consistently represented and genotyped across a large cohort of samples, we validate it using Mendelian inheritance, Hardy Weinberg statistics and other approaches. The resulting sets of high-quality variant annotations are essential for clinical applications where the variant calling is performed on one or few samples.

Our methods and visualisation tools will be made available as a part of our graph-based toolset for structural variant calling (https://github.com/illumina/paragraph). The validated calls and raw sequence data will be made available publicly for over 210 samples that have been sequenced to at least 30x depth as part of the Platinum Genomes (https://github.com/illumina/platinumgenomes) and Polaris (https://github.com/illumina/polaris) resources.

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P16.66B
Population-scale discovery of structural variants with PacBio SMRT sequencing

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Most of the base pairs that differ between human genomes are in structural variants (differences ≥50 bp), which are broadly missing from variant databases built with short-read DNA sequencing. To support use of structural variants in rare disease and common trait association studies, it is necessary to perform population-scale surveys with a technology effective at detecting structural variants, such as PacBio SMRT sequencing. After sample collection, the challenges to constructing population-scale structural variant databases are: 1. library preparation; 2. data collection; and 3. data processing. For library preparation, we developed SMRTbell Express Template Preparation, a 3-hour single-tube protocol. For data collection, prior studies have demonstrated that low coverage per sample (~5-fold) is sufficient for surveys of variation. The PacBio Sequel System generates low-coverage for a single sample in one day. For data processing, we developed pbsv, the first joint structural variant caller which combines reads from multiple individuals to identify variants. We first applied these tools to evaluate a human trio. At 5-fold coverage per sample, we discover most of the variants found by high coverage sequencing, with a 25% increase in sensitivity from joint variant calling. We further applied joint variant calling to a Mendelian disease case and identified a pathogenic, de novo structural variant. Finally, we developed a mathematical model to suggest how to power studies that apply 5-fold coverage to discover structural variants at a desired population frequency.Together, the tools and model support the creation of the first population-scale database of human structural variation.

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P16.67C

Prenatal and postnatal molecular characterization by Whole Genome Sequencing (WGS) of *de novo* structural variations (SVs)

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In the pre- and postnatal periods, *de novo* structural variants (SVs), such as supernumerary chromosomal markers (sSMCs) and apparently balanced translocations and inversions, are usually evidenced by standard karyotype. The presence of these SVs raises, especially during pregnancy, a difficult issue of prognosis due to the risk of intellectual disability. The advent of new technologies such as Whole Genome Sequencing (WGS) makes it possible to consider the detection and characterization of SVs. The goal is to use the WGS to identify and characterize SVs, and to secondarily improve our pipeline. A short-reads WGS was performed in 19 patients with *de novo* SVs (8 translocations, 7 complex chromosomal rearrangements (CCRs) and 4 sSMCs) that had been found in postnatal (11/19 patients) or prenatal (8/19 patients) periods. Bioinformatic analysis of the raw data was performed with Lumpy algorithms for translocations and inversions, and ControlFreec for CNVs. The analyses identified 7/8 translocations, one of which interrupts the *GRIN2B* gene at a breakpoint compatible with the patient's phenotype. The undetected translocation involves pericentromeric breakpoints. The 7 CCRs were also identified with, sometimes, the discovery of more than 10 breakpoints, in favor of a chromothrypsis type mechanism. Of the 4 sSMCs, the 2 with euchromatin were detected since the 2 with heterochromatin were not. This project allowed us to improve our WGS pipeline and identify SVs. It so demonstrates that short-reads WGS is particularly effective in characterizing the breakpoints of SVs (16/19) in both prenatal and postnatal periods.

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P16.68D

Open Targets: Integrating genetics and functional biology to identify and prioritise gene targets for disease

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Over the past decade, the genetic aetiology of many human diseases and complex phenotypes has been deeply characterised. However, attributing causal genes to genetic associations and developing effective therapeutics remains challenging.

In Open Targets, we integrate large-scale genetics and genomics with drug information to influence the way drug targets are identified and prioritised. We also generate new data using human cell models (e.g. organoids, iPSCs) and genome editing (CRISPR/Cas9) to identify drug targets for three main therapeutic areas: oncology, immunology, and neurodegeneration. The Open Targets Platform enables users to identify and investigate links between genes, pathways, and diseases, and can be accessed via a web interface or REST-API. We compute, score and rank target-to-disease associations using biological evidence integrated from the NHGRI-EBI GWAS Catalog, Genomics England, PheWAS, ClinVar, expression and eQTL resources, UniProt, ChEMBL, and many others. We have recently developed POSTGAP, a pipeline which merges common genetic associations curated from literature with functional genomics, epigenetic and expression data to resolve the association signals at each trait-associated locus and link each variant to its target gene(s). Functional evidence from multiple sources including GTEx data, promoter capture Hi-C data, DNase hypersensitivity sites, and FANTOM5 is incorporated into an evidence score, providing a single point of reference to explore the underlying biology of conditions and prioritise potential targets.

Aggregation and integration of functional data and annotation from multiple heterogeneous sources in Open Targets offers a robust solution to prioritising genes, quantifying their biological significance, and assessing their potential as pharmaceutical targets.

E.M. Schmidt: None.

P16.69A

Context-specific prioritization of non-coding variants implicated in human diseases

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Whole genome sequencing is increasingly being used for patients with rare genetic diseases as a diagnostic tool. However, for a large proportion of sequenced patients, no coding mutation is found in a gene associated with the phenotype. In these cases, a non-coding mutation, located in a cis-regulatory region, may affect the expression of a gene involved in the disease. Despite the existence of methods for annotating and predicting regulatory sequences on the basis of biochemical and epigenetic properties, defining objective criteria remains difficult to effectively select candidates among the millions of non-coding mutations present in each patient. Moreover, the mechanisms of action and interaction between regulatory regions and target genes are still unclear, making it difficult to link a non-coding mutation with the patient's phenotype.

We propose here a supervised machine learning strategy using random forests, adapted to complex and heterogeneous datasets, to classify and select non-coding mutations potentially involved in the deregulation of disease genes. A notable innovation of our approach is to take into account association data between non-coding regions and target genes.

We apply 3 classifiers, trained on different sets of experimentally predicted regulatory regions, on more than 40,000 non-coding mutations in 48 patients affected with X-linked intellectual disabilities from the FP7-funded project "NeuroXsys". Selected mutations were shown to segregate with the disease in affected families, and to deregulate the predicted target gene in animal models. We discuss the results in light of their genome-wide application to larger cohorts of patients.

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P16.70B

ExpansionHunter: A software tool to detect long repeat expansions from PCR-free whole-genome sequence data

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Introduction: Identifying large repeat expansions such as those that cause amyotrophic lateral sclerosis (ALS) and fragile X syndrome (FXS) is challenging for short-read (100-150 bp) whole genome sequencing (WGS) data. A solution to this problem is an important step towards integrating WGS into precision medicine. To this end, we developed a software tool called ExpansionHunter that, using PCR-free WGS short-read data, can genotype repeats at a locus of interest even if the expanded repeat is larger than the read length.

Materials and Methods: We applied ExpansionHunter to a set of 144 samples harboring repeat expansions associated with Huntington's disease, fragile X syndrome, Friedreich's ataxia and five other genetic disorders. In addition, we tested the C9orf72 repeat in a cohort of 3,001 ALS samples and compared our calls with RP-PCR results.

Results: All but one of the repeat expansions in the Coriell samples were detected by our method even though many of the repeats were significantly longer than the read lengths. In the ALS cohort, ExpansionHunter correctly

classified all (212/212) of the expanded samples as either expansions (208) or potential expansions (4). Additionally, 99.9% (2,786/2,789) of the wild type samples were correctly classified as wild type by our method with the remaining three identified as possible expansions.

Conclusions: ExpansionHunter now enables researchers to identify many known pathogenic repeat expansions from whole genome sequencing data.

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P16.71C
Cost-effectiveness of whole exome sequencing to solve the unsolved: an Italian pilot study

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Introduction: Rare, ultrarare and orphan diseases are heterogeneous disorders with a prevalence of less than 1/2000 persons, affecting some hundred millions of people worldwide. The needs of these patients and their families represent a medical, economical and societal challenge. The wide application of next generation sequencing has allowed a dramatic increase in the speed of genome sequencing with a direct impact in terms of diagnostic rate and a decrease in cost.

Materials & Methods: 300 patients were enrolled between 2014 and 2017 in the Undiagnosed Patients Program at Bambino Gesù Children Hospital (OPBG) in Rome. Each subject was suspected to have a mendelian disorder and remained undiagnosed despite extensive multidisciplinary clinical and instrumental evaluation, and targeted genetic testing. The cost-effectiveness analysis was performed on 211 patients, aged between 1 months and 43 years, w/wo a conclusive diagnosis following mendeliome/WES analysis. The total costs and costs for each year of diagnostic delay for the National Health System were compared with the cost of mendeliome/WES analysis.

Results: The average total cost for each undiagnosed patient was 11,572 € (range 160 – 75,840 €) and was related to complexity/severity of the disease to the patient's age. The estimated cost of each year of diagnostic delay was 2146 € (range 48 – 18,320 €).

Conclusions: While a subset of individuals affected by rare genetic diseases receives a diagnosis at the first clinical evaluation, a significant number remains undiagnosed. This study substantiates the cost-effectiveness of WES as a first line diagnostic tool in these patients.

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P16.72D
Orthogonal assessment of GENALICE MAP population calling accuracy using a cohort of 142 whole exome sequencing samples

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With a growing use of Next Generation Sequencing (NGS) in human genetics it is evermore important to benchmark data analysis pipelines. Benchmark datasets exist, such as NIST-GIAB and Illumina's Platinum Genomes, holding "truth" variants to determine sensitivity and specificity of NGS analysis pipelines. Although highly valuable, those benchmarks have drawbacks as NGS data analysis pipelines determined their truth calls. In addition, they include only a few individual samples that could lead to overfitting through training sets. Such drawbacks introduce biases that should be avoided in benchmark studies. Ruark et al (2016) have developed high quality whole exome sequencing data from 142 samples together with Sanger sequencing data at 730 loci (ICR142). This dataset allows NGS pipeline evaluation with orthogonal verified truth variants across a cohort of unrelated samples and avoids biases associated with other benchmark sets. We used the ICR142 dataset to validate the GENALICE MAP population calling tool. We observed correct calling of all 123 true SNPs and 260 out of 268 true INDELs. In addition, it called 6 SNPs and 18 INDELs at the remaining Sanger negative loci. We compared the results to those obtained with the bcbio platform (https://github.com/bcbio/icr142-validation) and observed that GENALICE MAP produces the lowest numbers of false negative and false positive calls. This makes GENALICE MAP best in class next to being extraordinarily fast: it took less than 3 minutes per sample to go from FASTQ to VCF.

Ruark et al. F1000Research 2016, 5:386 (https://doi.org/10.12688/f1000research.8219.1)

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Karten: A. Employment (full or part-time); Significant; Genalice Core BV.

P16.73A
QIAGEN Clinical Insight - Interpret (QCI-I) drives rapid identification and interpretation of candidate causal variants in two Personal Genome Project cases

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Introduction: Gathering the most current and accurate information is critical to variant interpretation and classification. The QIAGEN knowledgebase includes the most comprehensive database of variant specific publications, and data from public and proprietary databases. This resource is the cornerstone of QIAGEN Clinical Insight – Interpret (QCI-I), which facilitates rapid variant filtering, interpretation, and reporting. Here, QCI-I quickly identifies candidate causal variants in two cases from the Harvard Personal Genome Project (PGP).

Materials and Methods: Health records and VCFs were obtained from PGP (https://my.pgp-hms.org/users). Case 1 presented with dilated cardiomyopathy and case 2 exhibited autosomal dominant non-syndromic hearing loss. Filtering was performed in QCI-I to exclude poor quality bases, common variants, and those classified as benign or likely benign. VCFs were uploaded to QCI-I and respective phenotypes were entered.

Results: Case 1 harbored a single candidate causal variant in *LMNA* (c.176T>G), which was classified as likely pathogenic based on several lines of evidence in the QIAGEN knowledgebase. Review of curated publications revealed that this *LMNA* variant is novel in the context of cardiomyopathy. Upon analysis of case 2, QCI-I identified a compelling candidate causal variant in *HOMER2* (c.587G>C). While this variant is classified as a variant of uncertain significance, *HOMER2* has been mutated in autosomal dominant non-syndromic hearing loss previously.

Conclusions: Driven by content, QCI-I rapidly filtered VCFs and identified candidate causal variants in two PGP cases in under an hour. This work also highlights the importance of collaborative research efforts like PGP in furthering our understanding of genetics and disease.

J.L. Poitras: None.

P16.74B
GARFIELD-NGS: Genomic vARiants FIltering by dEep Learning moDels in NGS

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Motivation: Exome sequencing approach is extensively used in research and diagnostic laboratories to discover pathological variants and study genetic architecture of human diseases. Even if present platforms produce high quality sequencing data, false positives variants remain an issue and can confound subsequent analysis and results interpretation. Here, we propose a new tool named GARFIELD-NGS (Genomic vARiants FIltering by dEep Learning moDels in NGS), which is based on deep learning models to dissect false and true variants in single sample exome sequencing experiments performed with Illumina or ION platforms.

Results: GARFIELD-NGS consists of 4 distinct models tested on NA12878 gold-standard exome variants dataset (NIST v.3.3.2): Illumina INS/DELs, Illumina SNVs, ION INS/DELs, and ION SNVs. AUROC values for each variants category are 0.9269, 0.7998, 0.9464, and 0.9757, respectively. GARFIELD-NGS is robust on low coverage data down to 30X and on Illumina two-colour data, as well. Our tool outperforms previous hard-filters, and calculates for each variant a score from 0.0 to 1.0, allowing application of different thresholds based on desired level of sensitivity and specificity. GARFIELD-NGS processes standard VCF file input using Perl and Java scripts and produces a regular VCF output. Thus, it can be easily integrated in existing analysis pipeline.

Availability: GARFIELD-NGS available at https://github.com/gedoardo83/GARFIELD-NGS

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P16.75C
WGS based workflow for identification of disease causing variants in rare diseases

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Background: We have implemented a healthcare region-wide strategy for diagnosing patients with rare genetic disorders using WG (>3500 samples). This is result of collaboration between healthcare (Karolinska University Hospital) and academic settings, represented by Science for Life Laboratory.

Results: Approximately 100 samples are processed monthly. Turnaround time from DNA to results ready for clinical interpretation is 11 days (range 4 to 16). Using NIST samples, we estimate that SNV in known disease-causing genes are detected at >99.7% sensitivity and with >99.6% PPV. Indel detection is at 94% sensitivity. Detection of CNV and other SV is achieved using multiple variant callers. An in-house established frequency database is used to filter out technical artifacts.

Variants are ranked according to their disease causing potential using a rank sum model considering various sources of annotation, including observed inheritance pattern vs. expected pattern, CADD score, frequency, quality, consequence, clinical significance and other sources.

A suite of informatics solutions consisting of Chanjo (QC tool), Genmod (variant prioritization), and Scout (GUI), enable clinical experts without in-depth bioinformatic knowhow to evaluate the results.

The workflow is quality assured using ISO17025. Data sharing is being established through ClinVar and Beacon. Integration with multi-omic data is being evaluated for assisting in functional interpretation of clinical significance of variants outside coding regions and linked canonical splice sites.

In summary, we have introduced at large scale a healthcare region wide implementation of WGS for patients with rare genetic diseases. A key aspect has been the collaboration between academia and healthcare.

V. Wirta: None.

P16.76D
Disease interpretation of variants in the WGS dark matter: enhancers and ncRNAs

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Introduction: Whole genome sequencing (WGS) identifies 50 times more variants than exome sequencing, most residing in the genomic non-coding "dark matter". Three classes of functional genomic elements that thus become amenable to variant analyses are promoters, enhancers and ncRNAs, all central to tissue-related gene expression. Together they amount to >20% of the new DNA territories. Enhancers and some ncRNAs moderate spatiotemporal orchestration of embryonic development and cell differentiation, with many underlying diseases. The WGS challenge is disease interpretation of this new avalanche of dark matter variants.

Materials and Methods: We created GeneHancer (PMID:28605766), a novel regulatory element database with >250,000 enhancers and promoters. Information is amalgamated from six sources: ENCODE, Ensembl, FANTOM5, VISTA, dbSUPER and EPDnew. Target genes are gleaned via GTEx expression QTLs, Capture Hi-C, expression correlation between genes and FANTOM enhancer-transcribed ncRNAs, expression correlation of genes to enhancer-contained transcription factors, and genomic distance. In parallel, >100,000 unified ncRNAs are consolidated from 21 general and specialized databases (PMID:23172862).

Results: The widely-used GeneCards Suite has now been remodeled, creating an indispensable WGS disease interpretation platform. This is based on the abovementioned data and on >20,000 deeply annotated disease entries in MalaCards (PMID:27899610). This knowledgebase feeds the Suite's NGS tools: VarElect, the phenotype interpreter, and TGex, the VCF-to-report analyzer (PMID:27357693), augmented to prioritize WGS variants with respect to diseases and phenotypes.

Conclusions: The GeneCards Suite provides a comprehensive route to clinical significance of variants, coding and non-coding single nucleotide and structural genomic variations, often elucidating unsolved cases.

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P16.77A
Yield of Clinically Relevant Candidates in Family Genomes in the UK 100,000 Genomes Project Using the Fabric Genomics Platform

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The 100,000 Genomes Project, spearheaded by Genomics England (GeL), is a UK National Health Service sponsored study aimed at identifying disease-causing genetic variants in patients and families with rare genetic diseases and cancer using a WGS approach. For this study, clinical history was used to recruit patients into specific disease categories, each of which were associated with gene panels curated in the GeL PanelApp tool.

Fabric Genomics, a clinical interpretation partner for the 100,000 Genomes Project, has analyzed over 1220 clinical cases using Opal Clinical. The variant filtering and prioritization protocols utilized for case analysis include GeL's variant tiering methodology, ClinVar, and Fabric Genomics' proprietary variant and gene ranking algorithms VAAST (Variant Annotation, Analysis and Selection Tool) and Phevor (Phenotype Driven Variant Ontological Re-ranking Tool). We report results showing that by applying VAAST and Phevor we increase the clinical candidate yield compared to using the GeL tiering system alone. We identified candidate causal genes/variants in 42.3% of the cases. In 23.3% of these cases (9.8% overall) candidates were only obtained by using the VAAST/Phevor top 20 ranked genes/variants.

In a small subset of ~300 cases, we reviewed the effects of providing parental genomes in the analysis and return rate of results. Interestingly, there was a small difference in clinical candidate yield between solo cases and trios. Further analysis will be carried out to see if this difference is observed in a larger cohort.

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P16.78B

Phenotype-driven variant prioritization significantly improves over impact and prevalence scores in a large-scale analysis of 1,963 cases of Mendelian disease diagnostics by whole-genome sequencing

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Next-generation sequencing of genomes or exomes is becoming pervasive in the clinical diagnostics of Mendelian syndromes, idiopathic disease and fast diagnostics for newborns. Pressures remain to demonstrate the clinical utility of WGS and to reduce the cost of the diagnostic process. Traditionally, analysis consists in iteratively performing filtering steps using predicted impact and variant population prevalence and reviewing literature for candidate variants. We have previously developed probabilistic approaches to integrate variant impact and population prevalence (VAAST), and in combination with patient phenotype, leveraging the HuPO hierarchy (Phevor), to provide scores to prioritize variants for review clinicians. Here we present a large-scale analysis of 1,963 reviewed clinical WGS diagnostics cases allowing to quantify the performance of this approach. Probands where individuals with rare genetic diseases that failed single-gene based diagnosis. We identify the VAAST and Phevor score rank for 644 candidate causative variants ultimately selected by clinical reporting. Reports were generated with Opal Clinical software, which provides a rich set of annotations and both filtering widgets and VAAST and Phevor scores. We show that candidate causative variants reported reside in the top 10 VAAST rank in 35% of cases, whereas they are found in 70% of the cases when ranked by Phevor. When the candidate causative variant fell out of the top 20 rank, fewer and more ambiguous provided phenotypes are observed. In summary, the use of phenotype-driven variant prioritization scores can reduce significantly the interpretation turnaround time, ultimately reducing costs and increasing diagnostic rates.

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P16.79C

variant interpreter and genetic analysis summary generator

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We have developed a software that generates genetic analysis summary report by using VCF files are standard output files of variant identification programs. GenerAVI programmed by using java. It can be run on three must common operating systems: Windows, Linux, Mac-OSX. There is no installation requirement.

The software generates a genetic analysis summary report based on the evidence classification of the article of American College of Genetics and Genomics (ACMG) published for sequenced variants of clinical interpretation in 2015. Using 28 of evidence of ACMG, it classifies variants

into 5 groups as likely benign, benign, VUS, likely pathogenic and pathogenic. We have used 11 automatable evidences (PVS1, PM2, PP2, PP3, PP5, BA1, BS1, BS2, BP1, BP4, BP6) and an inhouse variant database for Turkish population to provide a genetic analysis summary report for MEFV, BRCA1, BRCA2 and CFTR genes.

Thus unbiased and reliable results can be generated by reducing the laborious time of variant curation to a minimum. Genetic analysis summary reports of 80 patients can be generated and be ready for review in a few minutes.

GenerAVI can use the manually curated variant database. Resources and data developed by Maxwell et al and by expert panels mentioned in ClinVar were used as a reference for variant classification. Also, users can interpret and store their variants. In addition, they can import variants interpretation of an expert panel into their database. GeneraAVI uses this variant database to generate genetic analysis summary report automatically.

Z. Gormez: None. **I. Erserim:** None. **D. Erdogan:** None.

P17 Epigenetics and Gene Regulation

P17.02B

Allele specific expression identifies rare variants as cause for extreme allelic imbalance

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Large eQTL studies have mostly been used to map the regulatory effects of common SNPs on gene expression. However, with current sample sizes it is difficult to identify eQTLs for rare variants. To address this we have used RNA-sequencing to genotype 4,001 blood samples from BIOS, a large Dutch biobank consortium, and used this to measure Allele Specific Expression (ASE) to assess the effects of rare variants on allelic imbalance.

Per gene we defined outlier samples as those that show an allelic imbalance that is 3 absolute deviations from the median. For the genes that have at least one outlier we find that there is an enrichment of rare variants within the outlier genes ($p = 2e\text{-}31$) and known pathogenic variants ($p = 4e\text{-}72$). When looking at high impact variants we observe that mendelian disease genes show stronger ASE effects in the case of nonsense mutations ($p = 1.66e\text{-}74$). We found that when a sample has a nonsense mutation in a mendelian disease gene this gene shows outlier ASE expression in 11% of the cases.

The results of this study show that rare variants are more likely to have a high impact and cause extreme allelic imbalance, underlining the importance of investigating allelic imbalance in the classification of variants of unknown clinical significance.

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P17.03C

Decreased methylation of the mitochondrial D-Loop region in peripheral blood DNA of amyotrophic lateral sclerosis patients

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Several evidences suggest that aberrant epigenetic mechanisms could be involved the etiology of amyotrophic lateral sclerosis (ALS). Particularly, methylation alterations in several nuclear genes have been detected in whole blood and spinal cord DNA of ALS subjects. Recently it has been reported also an involvement of epigenetic deregulation in mitochondrial DNA (mtDNA) in patients affected by neurodegenerative diseases, but evidences in ALS is limited.In the current study we investigated DNA methylation levels of the mitochondrial displacement loop (D-loop) region, which regulates mitochondrial DNA replication and transcription, in peripheral blood DNA samples collected from 40 ALS patients with mutations in one of the ALS associated genes, including *SOD1*, *FUS, TARDBP* and *C9ORF72*, and from 60 healthy individuals, some of which carriers of the same mutation. D-loop methylation levels were significantly lower in blood DNA of ALS patients than in healthy individuals ($P < 0.05$). Moreover a significant inverse correlation between D-loop methylation levels and mtDNA copy number ($r=-0.33$; $P < 0.001$), as well as a higher amount of mtDNA copy number in ALS patients compared to healthy individuals ($P < 0.001$)

were observed. Interestingly, when we considered methylation levels in relation to the mutations of ALS associated genes, lower D-loop methylation levels were detected in carriers of *SOD1* mutations, respect to carriers of mutations in the three other genes considered and non-carriers of mutations ($P < 0.05$).Present results indicate that mitochondrial D-loop region is hypomethylated in peripheral blood DNA of ALS patients and in carriers of *SOD1*mutations, and that this epigenetic signature is related to mitochondrial deregulation.

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P17.04D

***CHD8* suppression in neuronal progenitors correlates with alterations in chromatin landscape especially affecting transcriptional initiation and elongation**

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Autism Spectrum Disorders (ASD) is a collection of heterogeneous neurodevelopmental disorders defined by social impairment and repetitive behaviors with significant genotypic and phenotypic complexity. Mutations in several hundred loci have been associated with the disease, but the chromodomain helicase DNA binding protein 8 (*CHD8*) represents a recurrent and independently validated ASD-risk gene. All ASD mutations in *CHD8* gene have been reported to be disruptive, leading to haploinsufficiency. Here we investigate how chromatin landscape reacts to *CHD8* suppression by analyzing different histone modifications through Chromatin Immunoprecipitation and Sequencing (ChIP-seq) in iPS-derived neural progenitors after *CHD8* knock-down. We interrogated transcriptionally active and repressed regions as well as active and poised enhancers to explore the potential impact of *loss-of-function* mutations. *CHD8* suppression mildly affects the overall chromatin landscape leading to a generalized reduction in the number of peaks in actively transcribed (H3K36me3) and enhancer regions (H3K4me1 and H3K27ac). Particularly, transcriptional initiation and elongation chromatin states result the most significantly affected, highlighting genes implicated in "mitotic nuclear division", "cell cycle" and "mRNA processing" as the major dysregulated targets following *CHD8* suppression. Moreover, by overlaying histone marks data with *CHD8* binding sites, we observe that most chromatin changes seem to intervene in regions not-bound by *CHD8*, thus suggesting an '*indirect mode*' of chromatin remodeling. In summary, our results point toward broad regulatory consequence of *CHD8* suppression, possibly implicating altered RNA processing, as well as association with specific biological pathways of relevance to ASD pathogenesis.

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P17.05A

Female monozygotic twins phenotypically discordant for Beckwith-Wiedemann Syndrome sharing hypomethylation of IC2

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Beckwith-Wiedemann Syndrome is an overgrowth imprinting syndrome characterized by pre- and post-natal macrosomia, congenital abnormalities (such as macroglossia, omphalocele or umbilical hernia, visceromegaly, nephrourologic malformatios, ear anomalies) and tumor predisposition. The prevalence is about 1:10,500 live births, with an equal incidence in males and females, except for monozygotic twins, for which a significant female preponderance is reported. BWS in caused by defects at

chromosome 11p15.5 including Loss of Methylation (LoM) at the Imprintig Center 2 (IC2), Gain of Methylation at the Imprinting Center 1 (IC1), paternal uniparental disomy of 11p15.5 region and maternally inherited pathogenic variant of CDKN1C. In about 30% of BWS, MLID (Multilocus Methylation Imprinting Disturbance) is also reported. We present a case of monozygotic, monochorionic and diamniotic female twins discordant for BWS clinical manifestations (only one twin presented with macrosomia, macroglossia and nevus flammeus). At birth, the phenotypically affected twin had IC2 LoM and MLID on both blood and buccal smear samples, whereas the unaffected one showed IC2 LoM, without MLID, only on blood sample. Shared placental circulation and twin-to-twin transfusion have been hypotesized to explain the presence of epimutations in blood of the phenotypically normal twin. A two years clinical follow-up confirmed the complete absence of BWS signs in the unaffected twin, despite the maintenance of IC2 LoM in blood. We are currently investigating the methylation profile of placenta (collected at delivery) in order to deepen the insight into the epigenetic constitution of the fetal/placental compartment.

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P17.06B

Inhibition of histone deacetylation up-regulates the repressed paternal allele of the imprinted *Kcnk9* gene and improves the behavioral phenotype of a mouse model of Birk-Barel syndrome

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Kcnk9/KCNK9 is a maternally expressed imprinted gene, whose mutations are causative for the maternally inherited Birk-Barel mental retardation syndrome. It encodes a K2P-channel that controls resting membrane potential and excitability of neurons.

By analyzing WT, *Kcnk9*KO^{mat} and *Kcnk9*KO^{hom} mice in a behavioral test battery during the light phase, our data shows that the absence of *Kcnk9* leads to impaired working memory, reduced acoustic startle response and abnormal sensorimotor gating. Investigations of circadian rhythms revealed selectively increased locomotor activity during the dark phase in *Kcnk9*KO^{hom} and, to a significantly smaller extent, in *Kcnk9*KO^{mat} mice compared to controls. Using Quantification of Allele-Specific Expression by Pyrosequencing (QUASEP) and Allele-Specific RT-qPCR in wildtype (C57BL/6xCast/Ei)F1 hybrid mice, biallelic *Kcnk9* expression from the repressed paternal allele (1-17% of transcripts) was observed in all analyzed brain regions and was particularly strong in the locus coeruleus (LC). Slice patch-clamp recordings revealed wildtype-like pacemaker activity during the dark phase in LC neurons from *Kcnk9*KO^{mat} but not from *Kcnk9*KO^{hom} mice, which discharged at significantly higher frequencies. The neuronal data are in line with the locomotor phenotype and demonstrate the functional relevance of paternal *Kcnk9* expression.

Through epigenetic manipulation with CI994, a specific histone deacetylase inhibitor, we could induce a significant up-regulation of the paternal *Kcnk9* allele in several analyzed brain regions after injections in *Kcnk9*KO^{mat} mice. Together with this we observed a significant behavioral improvement of *Kcnk9*KO^{mat} mice after CI994 treatment. This novel approach shall open new avenues for treatment of cognitive dysfunctions in Birk-Barel syndrome and other imprinting disorders.

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P17.07C

Urine microRNA profiling by next generation sequencing and multiple mutations in urinary exfoliated cells in bladder cancer

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Bladder cancer (BC) is one of the most aggressive malignancies of the urinary tract. Because of the high rate of recurrences requiring continuous surveillance, BC is the most expensive cancer for the health system.The

identification of new biomarkers for early BC detection, recurrence/progression is urgently needed to both improve patient outcomes and decrease health care costs. MicroRNAs (miRNAs) are aberrantly expressed in BC, and may be isolated from various biological specimens, including urine. We performed a miRNA profiling in urine samples from 66 BC male patients (10 muscle invasive BC (MIBC) and 56 non-muscle invasive BC (NMIBC)) and 48 healthy controls using a Next Generation Sequencing approach that could accurately distinguish BC patients and predict disease outcome. Specific urinary miRNA signatures could distinguish the different types of BC patients from healthy controls in one of the best and closest surrogate tissue for BC. Twenty-three miRNAs (21 target and 2 reference miRNAs) were validated by qPCR on 177 urine samples from 113 BC case and 64 controls . MiRNA signatures were also evaluated in concomitance with the presence of a panel of mutations in DNA from exfoliated urinary cells from the same samples. Forty-one mutations in TERT, FGFR3, PIK3CA, and RAS were analyzed in relation to clinical outcome. We will present the results on the possibility to use urinary miRNAs and exfoliated urinary cell mutational status as diagnostic, prognostic and predictive biomarkers for BC.

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P17.08D

Breast cancer epigenetics: abnormal promoter methylation of matrix and transmembrane proteins encoding genes

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Extracellular matrix molecules and transmembrane receptors organize tissue structure and provide its adequate function. During cancer progression both these features suffer disruption.

Materials and Methods: We analyzed methylation status of 12 laminin-encoding genes (*LAMA1, LAMA2, LAMA3A, LAMA3B, LAMA4, LAMA5, LAMB1, LAMB2, LAMB3, LAMC1, LAMC2, LAMC3*), 8 integrins (*ITGA1, ITGA2, ITGA3, ITGA4, ITGA6, ITGA7, ITGA9, ITGB1*), 2 nidogens (*NID1,NID2*), 2 cadherins (*CDH2,CDH3*), the dystroglycan gene *DAG1* and 11 matrix metalloproteinases-encoding genes (*MMP2, MMP11, MMP14, MMP15, MMP16, MMP17, MMP21, MMP23B, MMP24, MMP25, MMP28*) and 4 tissue inhibitors of matrix metalloproteinases genes (*TIMP1, TIMP2, TIMP3, TIMP4*) in 206 breast cancer samples, 206 paired adjacent nonmalignant samples, 6 samples of normal mammary gland from autopsy and 6 samples of breast cancer cell lines by MSRE PCR and bisulfite sequencing.

Results: Promoters of 17 genes (*LAMA1, LAMA2, LAMB1, LAMC1 NID1, NID2, ITGA1, ITGA4, ITGA7, ITGA9, CDH2,CDH3, MMP2, MMP23B, MMP24,MMP25, MMP28)* have shown abnormal methylation in 3,4% to 41,5% samples of breast cancer and adjacent tissues.

Further statistical analysis with additional data have demonstrated, that methylation of 15 gens *LAMA1, LAMA2, LAMB1, NID1, NID2, ITGA1, ITGA4, ITGA7, ITGA9, CDH2,CDH3, MMP2, MMP24, MMP25, MMP28* was strongly associated with highly methylated breast cancer type. Based on this information we designed a system of markers which discriminate hyper- and hypomethylated subtypes of breast cancer with sensitivity and specificity of 0,79 and 0,78, respectively; AUC=0,83.

	Methylation in breast cancer and/or adjacent nonmalignant samples (%)	Methylation in normal mammary gland from autopsy (%)	Presence(+) / absence(-) of methylation in breast cancer cell lines						Association with clinicopathological features
			ZR	MCF7	T47D	BT	HBL	HS	
LAMA1	29,4	0	+	+	+	+	+	-	-
LAMA2	27	0	+	+	+	+	+	-	HER2=3+ Luminal B type
LAMB1	26	0	+	+	+	+	-	-	HER2=3+ Luminal B type
ITGA1	15,2	0	-	-	-	-	-	-	-
ITGA4	30	0	+	+	+	+	-	-	HER2=3+
ITGA7	3,4	0	-	-	-	+	-	-	-
ITGA9	41,3	0	+	+	+	+	+	-	Unmethylated status associated with triple-negative type, lack of ER
NID1	38,7	0	+	+	+	-	-	+	Unmethylated status associated with triple-negative type
NID2	41,2	0	-	+	+	+	-	-	-
CDH2	8,8	0	-	-	-	-	-	-	-
CDH3	41,5	0	+	+	+	+	+	-	Lack of ER
MMP2	7,7	0	+	+	+	-	-	-	-
MMP23B	17	0	+	+	+	-	+	+	Lack of ER, PR HER2=3+ HER2-positive type
MMP24	12	0	-	-	-	+	+	-	-
MMP25	15,4	0	-	+	+	-	+	-	-
MMP28	4,9	0	+	+	+	+	+	-	-

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P17.09A

Genetic variation on transcription factor binding sites nearby human genes associated with Brugada syndrome

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Genetic variation within transcription factor binding sites (TFBS) generates diversity in gene expression regulation, and may lead to increased or decreased risk for human disease. Here, we aim to characterize genetic variation at genomic locations candidate to host TFBS nearby genes associated with Brugada Syndrome (BrS). BrS is an arrhythmogenic disease that has been extensively associated with genetic variations in genes encoding cardiac ion channels (25-30%), but the contribution of genetic variation at nearby TFBS remains unknown. We integrated ENCODE data of topological organization, chromatin accessibility, histone marks, and transcription factor binding in human cardiac cells and annotated 1,300 genomic loci that potentially host TFBS nearby BrS-associated genes. To characterize genetic variation in these regions, we selectively captured and sequenced in depth (x100) this set of 1,300 loci in a cohort of 89 BrS cases (BrS cohort), and compared them with genetic variations in the Wellderly cohort (healthy-aging individuals). In the BrS cohort, we identified 5,382 single-nucleotide variants (SNVs) and 737 insertions and deletions (Indels). We observe 4,231 SNVs in common between both cohorts, while the remaining 1,888 SNVs are BrS patient-specific. Our current analyses are focused on the integration of these data to determine the potential simultaneous contribution of multiple TFBS to the disease. To investigate the potential functional relevance of our findings, we also integrate data of experimentally validated CTCF-TFBS, and machine learning-based predictions of TFBS for cardiac transcription factors. Collectively, our study represents the most exhaustive analysis up-to-date of genetic variation associated with BrS.

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P17.10B

Eye-specific transcription factor PAX6 and CRX regulate the expression of a *de novo* pyrimidine biosynthesis gene *Cad*

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Introduction: CAD is a tri-functional enzyme comprise the first three steps of total six steps of *de novo* pyrimidine biosynthesis with unknown role during mammalian development. Zebrafish *Cad* mutants exhibited reduced eye size, growth, tectum, jaw, and pectoral fins abnormalities and the gene is highly expressed the in the retina. Through a three-generation ENU program, two mouse *Cad* mutant families were generated: the *L5Jcs24* (missense mutation) and the *L5Jcs27* (nonsense mutation). Homozygotes were embryonic lethal, and heterozygotes exhibiting *small eyes* with degenerated retina. Due to the phenotypic similarities between well-studied eye-specific transcription factors, *Pax6* and *Crx*, we speculated that these genes might function in the same pathway in controlling ocular development. *In silico* transcription factor binding site analysis predicted multiple potential binding sites for both PAX6 and CRX.

Materials and Methods: Luciferase assay were used to evaluate whether *Cad* promoter is under transcriptional control of *PAX6* or *CRX*. We also use shRNA knockdown combined with Western blotting and qRT-PCR, to further evaluate our hypothesized that PAX6 and CRX might be involved in the regulation of *Cad* gene expression.

Results: The luciferase activities of Cad promoter were up-regulated by PAX6 and CRX for six-fold when transfected alone, and the promoter activities were up-regulated for 14 times when PAX6 and CRX were co-transfected to the Cad promoter.

Conclusions: We have confirmed that PAX6 and CRX bind to the promoter of Cad region and up-regulated gene expression in additive fashion.

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P17.11C

Developing CAR-T cells therapies : Is the European law adequate ?

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In December 2017, at the American Society of Hematology, the successful use of CAR-T cells in several specialized worldwide centres was presented. This therapy consists in the development of T cells (type of immune cells) in laboratory, inserting into them, the gene of a special receptor called chimeric antigen receptor (CAR). This manipulation will modify the immune cells, so that they carry the same target of cancer cells, that will be attacked. This method requires rigorous organisation since it should imply serious side effects. Thus, it is relevant to analyse the framework of this revolutionary method of treatment first concerning their legal qualification which defines the regulatory pathway to seek for marketing authorization and second the bioethical principles this innovation could challenge. According to the innovative characteristics of CAR-T cells, EU strictly regulates their use by considering them like gene therapy and the Committee for Advanced Therapies has provided specific guidance for their development. So professionals, using CAR-T cells should implement specific safety rules for the use of cells and for patients from sampling to the infusion of the modified cells. Moreover, professionals and medical establishments will have to adapt their facilities and to develop specific medical expertise to implement the technique in practice. Finally, beyond these technical aspects, the transformation of cells into a drug questions the bioethical principles beyond the use of the elements of the human body such as property, autonomy or justice The latter is of major importance due to the high cost of the treatments.

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P17.12D
Molecular bases of desogestrel effects in congenital central hypoventilation syndrome (CCHS)

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Introduction: The paired-like homeobox 2B gene (*PHOX2B*) encodes a key transcription factor that plays a role in the development of the autonomic nervous system. In humans, its over-expression is associated with the pathogenesis of neuroblastomas, and heterozygous *PHOX2B* mutations cause Congenital Central Hypoventilation Syndrome (CCHS), a life-threatening neurocristopathy characterised by failure of autonomic respiratory control. A partial recovery of ventilation has been observed in some CCHS patients using the potent contraceptive progestin desogestrel. Moreover, previous findings have shown that progesterone suppressed the growth of neuroblastoma *in vitro* and *in vivo*.

Materials and Methods: To investigate the effects of desogestrel on the expression of *PHOX2B* and its target genes, we generated a progesterone-responsive neuroblastoma cell line.

Results: Our findings demonstrate that, through progesterone nuclear receptor PR-B, desogestrel down-regulates *PHOX2B* gene expression, by a post-transcriptional mechanism, and its target genes, thus reinforcing the role that PHOX2B has in the pathogenesis of CCHS and in therapy response.They also further support the view that the drugs and molecules that are effective in counteracting neuroblastoma cell growth act through the down-regulation of *PHOX2B* gene expression, and open up the possibility that this mechanism may contribute to the positive effects observed in some CCHS patients.

Conclusions: These findings have a strong proof of concept value, in the perspective of a pharmacological intervention in CCHS, at least for ameliorating respiratory symptoms.

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P17.13A
Where SNPs, coexpression and TADs intersect: new insights in the regulation of coexpression in celiac disease

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Introduction: Alterations in expression and coexpression patterns have been shown in celiac disease (CD), but the mechanisms behind those changes remain unclear. Topologically Associating Domains (TADs) are functional domains of gene expression coordination and could explain those changes. Thus, the aim of this study is to identify SNPs that could be altering TAD structures and thus

deregulate (co)expression in CD, using data from coexpression experiments, location of TADs and CD-associated SNPs.

Materials and Methods: We used RNAseq data from the epithelial fractions of intestinal biopsies from 10 CD patients and 12 control individuals to build coexpression matrixes; next, we checked whether coexpressed genes overlapped with conserved TADs, and search for associated SNPs that could alter those TADs. A candidate region was deleted from HCT15 and 293FT cell lines using CRISPR-Cas9 technology, and the expression of neighbouring genes was assesed.

Results: Thirty-eight putative TAD fusions and disruptions were identified when we compared celiac and control groups and considered TAD and CD-associated SNP coordinates. Deletion of an inter-TAD region spanning chr20: 34026832 - 34027214 (Hg19), including a CTCF binding site and a DNase hypersensitivity site, located near the associated risk SNP rs224436 resulted in the coexpression of adjacent genes *PROCR* and *ROMO1* resembling active CD.

Conclusions: This study shows that changes in TAD structure could functionally explain part of the genetic associations in complex diseases through gene (co)expression regulation.

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P17.14B

Discovery and validation of altered methylation loci as early biomarkers of colorectal cancer

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Introduction: Colorectal cancer (CRC) develops through the accumulation of both genetic and epigenetic alterations. While the former are used as prognostic and predictive biomarkers, the latter are less characterized. The aims of the study were to identify signature alterations in the CRC methylome, to test whether they represent early events in CRC development and to explore the use of non-invasive techniques to reveal altered methylation.

Materials and Methods: methylome analysis was conducted by HumanMethylation450 BeadChips and raw data were analysed using RnBeads. Pathways enrichment was evaluated using ToppGene. *In silico* validation was performed in publicly available datasets. Methylation of three selected and validated markers was analysed in 24 stool DNAs and 45 plasma DNAs by digital PCR.

Results: we identified in 39 samples and validated in over 500 samples a panel of 74 altered CpG islands. The panel discriminates CRCs and adenomas from peritumoral and normal mucosa, with very high specificity (100%) and sensitivity (99.9%). To establish the usefulness of these findings as non-invasive markers for detection of CRC, we selected and tested three biomarkers in stool DNA and plasma cell-free circulating DNA, confirming the presence of altered methylation in affected patients.

Conclusions: our study identifies a panel of genes with strongly altered methylation in both adenomas and CRCs, candidating its use as biomarker for adenomas and early CRC detection through non-invasive techniques.

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P17.15C

GHSR methylation as a biomarker of colorectal cancer

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Introduction: Epigenetic events contribute significantly to colorectal cancer (CRC) pathogenesis. DNA methylation signatures have been proposed as potential biomarkers in CRC diagnosis and in measuring response to therapy. Recently, hypermethylation of the growth hormone secretagogue receptor (*GHSR*) gene has been proposed as a common epigenetic alteration of high diagnostic value in a broad spectrum of cancers. Regarding CRC, only a single study addressed this issue in a small sample of CRC specimens.

Materials and Methods: In order to validate *GHSR* hypermethylation as a CRC biomarker we compared 73 DNA samples extracted from CRC tissues and 73 DNA samples obtained from the healthy colonic mucosa of the same patients.

Results: Very interestingly we observed a statistically significant hypermethylation of *GHSR* in tumor tissues than in healthy mucosa (51.3% vs. 20.5%, $P < 5 \times 10^{-7}$). ROC analysis revealed a high degree of both sensitivity and specificity for discriminating tumor tissue and corresponding healthy mucosa (AUC value of 0.819). No correlation between *GHSR* hypermethylation in tumor DNA samples and tumor stage, size, location, or patient's age and gender was observed, indicating that this is an early epigenetic event already observable at the adenoma stage. Moreover, no correlation between *GHSR* methylation and polymorphic variants of genes involved in DNA methylation reactions, including DNA methyltransferases and folate-related genes, was observed. In the normal colonic mucosa tissue a significant positive correlation between *GHSR* methylation and age (r=0.34; P=0.003) was observed.

Conclusions: Present results confirm *GHSR* methylation as a strong and highly accurate epigenetic biomarker of CRC.

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P17.17A
TAP1 and *HLA-B* genes in the HLA region are hypomethylated and overexpressed in celiac duodenal cell populations

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Introduction: The Human Leucocyte Antigen (HLA) explains around 40% of the heritability of celiac disease (CD). However, the pathogenesis of CD could be driven by other layers of genomic information independent from inherited sequence variation, such as DNA methylation, also in this region.

Methods: DNA methylation landscape is expected to be different among cell types. Therefore, we analyzed the methylome and the transcriptome of sorted epithelial and immune cells of duodenal biopsies in 10 CD-patients and 10 controls. Methylation differences were confirmed by next generation sequencing (NGS) in bisulfite-treated, PCR-amplified fragments spanning *TAP1* and *HLA-B* promoters in an independent cohort of 14 inactive-CD and 14 control individuals.

Results: *TAP1* was hypomethylated in the CpG island shores surrounding its promoter in both epithelial and immune cells, and so was *HLA-B* in the epithelia. This was confirmed in complete biopsies from inactive-CD patients. Additionally, *TAP1* and its overlapping gene *PSMB9* were overexpressed in the epithelial and immune fractions, while the upregulation of *HLA-B* and its overlapping pseudogene was observed only in epithelia. Both *TAP1-PSMB9* and *HLA-B*-pseudogene pairs were coexpressed.

Conclusions: The confirmation of the differential methylation in inactive-CD suggests that alterations were acquired early in life. Expression results are coherent with the cell type-specificity of the methylation alterations.We propose the identified genes as novel CD candidates and particularly *TAP1*, an important HLA class-I peptide whose exacerbated expression suggests an anomalous boost of adaptive immunity.

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P17.18B
Analysis of endothelin-1 (*EDN-1*) promoter region

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Endothelin-1 (ET-1) is a peptide secreted by the endothelium of blood vessels that promotes vasoconstriction. A deregulation in the synthesis of endothelin-1, increasing its secretion, is a triggering factor for Pulmonary Arterial Hypertension (PAH). We carried out the characterization of the promoter region of endothelin-1gene (*EDN-1*), in order

to determine possible variations that may be associated with this disease and therefore target possible treatments.

The genetic analysis was carried out in 13 patients with Idiopathic PAH (IPAH), analysing a fragment of 2 kb promoter region. First, an *in silico* analysis was performed to evaluate binding transcription factors and CpG islands. Sequencing data was aligned to the reference Ensembl *EDN-1* sequence. Luciferase assay was done to evaluate *in vitro* the SNP influence in gene expression.

A deletion in the promoter region was found (rs397751713). The distribution of the genotype frequencies in our IPAH patients were: A/A: 0.15; A/-: 0.31; -/-: 0.54. This variation is located in a KLF4 binding sequence, a transcription factor related to PAH development. A CpG island was also detected that comprise the aforementioned variation. Future methylation pattern studies will be performed.

In conclusion, this SNP in the promoter region of *END1* could be related with gene expression levels. Even more, the epigenetic regulation could be also related to the methylation state of this region. All these data have to be taken carefully as these are preliminary data from 13 patients. Deciphering the regulation of *EDN1* expression could shed light in the molecular basis of this disease.

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P17.19C

Epigenetic role of DNA transposable elements (TEs) in shaping CD4+ T cell identity and plasticity in health and disease

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CD4+ T lymphocytes coordinate adaptive immune responses differentiating into functionally different subsets that exert an extraordinary degree of functional plasticity, novel epigenetic players are still being searched to explain immune related phenotypes. Notably, among non coding genome, Transposable Elements account roughly for the 45% of the genome and although ignored for decades, are nowadays robustly emerging as novel key molecules acting at different level of cell type specific genome regulation.We are dissecting TEs function in the epigenetic regulation of human primary CD4+ lymphocytes. We found that CD4+ T cells subsets show a specific and dynamic expression of LINE1 (L1) elements, being Naïve and Treg enriched in chromatin associated L1 transcripts in respect to T effectors, that are less enriched (i.e. Th1, Th2). Interestingly, L1 RNAs show a peculiar and timely specific dynamics being rapidly depleted from the nuclei in a TCR activation dependent manner. Furthermore, chromatin associated L1 RNAs localize on genomic regions different from L1 DNA, enriched in euchromatic marker (e.g. H3K4me3, H3K36me3) avoiding heterochromatin (e.g. H3K9me3), foreseeing a possible ncRNAs regulatory function. We have also collected preliminary observations on CD4+ T naïve cells derived from new born and old individuals, indicating that L1 transcriptional deregulation is a hallmark of immune system aging. We are now dissecting with novel, custom Next Generation Sequencing technologies L1 relevance at transcriptional and genomics level in order to dissect the mechanisms by which L1 elements might contribute to human CD4+ T lymphocytes cell identity, plasticity and specialization in healthy and disease conditions.

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P17.21A

Using ChIP-Seq to Profile FFPE Preserved Tumors for Epigenetic Alterations

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FFPE preserved tumors are a valuable resource for retrospective research on clinical samples. Clinical information, treatments and outcomes are often available for these samples providing an opportunity to link molecular biology research data to disease, diagnosis and biomarker discovery. Unlike fresh tumor samples, which have been used in ChIP-Seq-based epigenomic profiling experiments to discovery novel cancer subtypes, FFPE samples are more challenging due to extensive crosslinking which hampers chromatin extraction. However, recent advances at Active Motif have enabled the routine generation of high quality ChIP-Seq data sets from limited amounts of FFPE preserved tumors. FFPE ChIP-Seq data shows 1) extracted chromatin can be used with multiple different histone modification specific antibodies resulting in the expected genome-wide pattern for each histone mark 2) FFPE ChIP-Seq epigenetic profiles are highly concordant with profiles generated from matched freshly frozen tumors 3) FFPE ChIP-Seq profiles reveal tumor specific, differential histone occupancy patterns 4) More challenging transcription factor targets, such as Estrogen Receptor, can be profiled efficiently using FFPE ChIP-Seq. It is anticipated that the availability of a reliable ChIP-Seq method for profiling FFPE preserved patient samples will result in advancements in our understanding of

disease, lead to the discovery of new biomarkers and eventually impact therapeutic decisions.

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P17.22B

chromatin landscape of D4Z4 repeat interactome unveils a muscle atrophy signature in facioscapulohumeral dystrophy

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Despite increasing insights in genome structure organization, the role of DNA repetitive elements, accounting for more than two thirds of the human genome, remains elusive.Facioscapulohumeral Dystrophy (FSHD) is associated with deletion of D4Z4 repeat array below 11 units at 4q35.2. It is known that the deletion alters chromatin structure *in cis*, leading to gene upregulation. Here we show a genome-wide role of 4q-D4Z4 array in modulating gene expression via 3D nuclear contacts. We have developed an integrated strategy of 4q-D4Z4 specific 4C-seq and chromatin segmentation analysis, showing that D4Z4 3D interactome and chromatin states of interacting genes are impaired in FSHD; in particular, genes which have lost the D4Z4 interaction and with a more active chromatin state are enriched for muscle atrophy signature. Among these, we further characterized the muscle atrophy marker *Atrogin 1* (8q24), showing by 4C-seq and 3C strengthened enhancer-promoter chromatin loops at the locus and transcriptional upregulation during FSHD myogenic differentiation. Expression level of these genes is restored by an ectopic wild type 4q-D4Z4 array, suggesting that the repeat directly modulates the transcription of contacted targets.

Our study provides insight into the epigenetic role of DNA repeats in fine-tuning gene transcription by orchestrating the crosstalk between chromatin folding and structure, which deregulation maybe central in human genetic diseases pathophysiology.

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P17.23C

Development of a novel methylation based fetal fraction estimation assay using multiplex ddPCR

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Introduction: Accurate fetal fraction assessment is very important in non-invasive prenatal testing (NIPT). Affected samples with low fetal fraction have an increased risk for misdiagnosis. We present a multiplex droplet digital PCR (ddPCR) assay for fetal fraction estimation using methylation sensitive restriction enzymes (MSREs) and a robust set of novel fetal-specific differentially methylated regions (DMRs).

Methods: We discovered 38 fetal-specific DMRs which can potentially be used for fetal fraction estimation in NIPT. Eight biomarkers (7 DMRs and 1 reference control) were selected for further analysis. An assay comprising MSRE digestion followed by multiplexed (octaplex) ddPCR was developed for fetal fraction estimation. A chromosome Y-multiplex ddPCR assay (YMM) was also developed for fetal fraction estimation in male fetuses. YMM was used to test the robustness of the methylation-based fetal fraction estimation assay in 138 male pregnancy samples. A final validation was performed on 234 pregnancy samples.

Results: YMM was used to train the methylation-based fetal fraction estimation model. Statistical analysis resulted in the final optimal methylation assay which employs 4 DMRs (FFMM). High correlation between FFMM and YMM fetal fraction measurements was observed in 85 male pregnancies (r=0.86 CI:0.80-0.91) and confirmed using additional 53 male pregnancies. A final validation on 234 pregnancies using FFMM and fetal fraction measurements obtained from the VERACITY NIPT test showed strong correlation.

Conclusions: We developed a robust methylation-based assay for accurate fetal fraction estimation using a novel set of fetal-specific DMRs. This simple method can be used as an accurate fetal fraction estimation tool in NIPT.

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P17.24D

Allele specific chromatin signals, 3D interactions, and refined motif predictions for immune and B cell related diseases

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Introduction: Several Genome Wide Association Studies (GWAS) have reported variants associated to immune system diseases. However the identified variants are rarely the real drivers of the associations due to the heterogeneity in and between the study groups and most of the molecular mechanisms behind the genetic contributions to immune diseases remain poorly understood. ChIP-seq data for TFs and histone modifications provide snapshots of protein-DNA interactions allowing the identification of heterozygous SNPs with significant allele specific binding (AS-SNPs). These variants, which can affect a TF binding site resulting in altered gene regulation, are primary candidates to explain associations observed in GWAS and expression studies.

Results: We identified 17,293 unique AS-SNPs across 7 lymphoblastoid cell lines of which 237 were associated to immune GWAS traits and 714 to gene expression in B cells. To elucidate possible regulatory mechanisms we integrated long-range 3D interactions data (HiC and HiCap) to identify putative target genes and motif predictions based on Parsimonious Markov Models (PMMs) to identify TFs whose binding may be affected by AS-SNPs yielding a collection of 173 AS-SNPs associated to gene expression and 60 to B cell related traits.

Conclusions: We present a systems strategy to find functional gene regulatory variants, the TFs that bind differentially between alleles and novel strategies to detect the regulated genes.

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P17.26B

Transcription-dependent *de novo* DNA methylation at the imprinted *Zrsr1*-DMR occurs in the growing oocyte, but not in early embryonic cellsH. Soejima[1], F. Matsuhisa[1], S. Kitajima[1], K. Nishioka[1], K. Higashimoto[1], H. Yatsuki[1], T. Kono[2], H. Koseki[3], K. Joh[1];

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Zrsr1 is a paternally expressed imprinted gene located in the first intron of *Commd1*, and the *Zrsr1* promoter resides in a differentially methylated region (DMR) that is maternally methylated in the oocyte. *Commd1* is transcribed in the opposite direction to *Zrsr1* and shows predominant expression of the maternal allele, especially in the adult brain. A mechanism for the establishment of methylation at *Zrsr1*-DMR in the oocyte has not been well established. *Commd1* is expressed in the growing oocyte; therefore, *Zrsr1*-DMR transcription occurs when methylation is established. *Zrsr1*-DMR methylation was abolished by inserting a poly(A) signal cassette into *Commd1*, which prevents transcription through the DMR. Methylation did not occur at the artificially unmethylated maternal *Zrsr1*-DMR during embryonic development when transcription at the DMR was restored by deleting the truncation cassette in the zygote. Loss of methylation at the maternal *Zrsr1*-DMR resulted in biallelic *Zrsr1* expression and reduced the extent of the predominant maternal expression of *Commd1*. These results indicate that the establishment of methylation at *Zrsr1*-DMR occurs in a transcription-dependent and oocyte-specific manner, and caused *Zrsr1* imprinting by repressing maternal expression. The predominant maternal expression of *Commd1* is likely caused by transcriptional interference by paternal *Zrsr1* expression.

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P17.27C

A study on global DNA methylation in white blood cells of patients with breast cancer

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Methylation changes have long been studied to trigger initiation and progression of tumors. The aim of our research was to compare changes in global methylation levels in blood cells from patients having invasive ductal carcinoma of breast with different histological parameters and stage.

20 patients with biopsy-proven invasive ductal carcinoma of breast and no pre-operative chemotherapy were randomly selected for the study. Blood was collected preoperatively. Global DNA methylation was quantitatively measured using ELISA-based assay. Post-operative specimen was processed routinely for investigation of histological characteristics.

The results showed that methylation level in genomic DNA from white blood cells correlated neither with the grade nor the stage of tumor. There was no relationship of global DNA methylation level with the size or histological parameters (duct formation, nuclear pleomorphism, mitotic activity) of the tumor, nor with lymph node status. The only variable found do be related with methylation level was the age - patients older than 75 showed to have significantly higher global methylation level compared to patients under 50.

The results support our previous investigation data showing tumor-specific methylation changes (like LINE-1 methylation) to be much better pronounced in tumor tissue and normal ductal epithelial cells from adjacent area rather than in blood. Epigenetic changes in cancer tissue and normal breast tissues will further be studied in this group to get a better understanding of whether or not global or site-specific methylation plays a role in determining histopathological characteristics of cancer.

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P17.28D

Persistent effects of urate on cytokine production with implications for epigenetic regulation in gout

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Introduction: Metabolic triggers are important modulators of inflammation in gout. Hyperuricemia predisposes to monosodium urate (MSU) crystallization and also primes inflammatory responses in relation to TLR stimulation. Genetic studies have identified 28 loci associated to gout mainly based on urate outcomes, whereas inflammatory and epigenetic factors are potential additional factors in the gout phenotype.

Materials and Methods: Monocytes from healthy volunteers were pre-treated with urate for 24h and then subjected to increasing resting periods, followed by 24h stimulation with TLR2 or TLR4 ligands in the presence or absence of MSU. Cytokine production was assessed by ELISA. ChIP sequencing was performed in treated cells.

Results: We show in in vitro and in vivo studies that high levels of uric acid enhance inflammation and that broad spectrum methylation inhibitors reverse uric acid effects. The higher IL6 and lower IL-1Ra production persisted for up to 3 days after treatment. Genome wide assessment of two main candidate histone posttranslational modifications (histone 3 lysine 4 trimethylation, H3K4me3, and histone 3 lysine 27 acetylation, H3K27ac) did not show significant differences in the epigenetic landscape for these marks after in vitro urate treatment in human monocytes.

Conclusion: Our data suggests the involvement of methylation regulatory pathways in response to uric acid and excludes H3K4me3 and H3K27ac as regulatory marks. Future perspectives for genetic studies using hyperuricemic controls are warranted for gout research, while the exploration of urate induced epigenetic regulation is likely to help understand the variability in the gout phenotype.

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P17.29A

Variation in microbiome composition impacts human gene expression by changing chromatin accessibility

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Variation in gut microbiome is associated with human disease, yet the underlying molecular mechanisms are not well understood. A likely mechanism is through changes in gene regulation in interfacing host epithelial cells. Here, we treated colonic epithelial cells with live microbiota from five healthy individuals and quantified changes in transcriptional regulation and chromatin accessibility in host cells. We

identified over 5,000 host genes that change expression, including 588 distinct associations between specific taxa and host genes. The taxa with the strongest influence on gene expression alter the response of genes associated with complex traits. Further, using ATAC-seq, we show that these changes in gene expression are likely the result of changes in host chromatin accessibility induced by exposure to gut microbiota. We then created a manipulated microbial community with titrated doses of Collinsella, demonstrating that both natural and controlled microbiome composition leads to distinct, and predictable, gene expression profiles in the host.Together, our results suggest that specific microbes play an important role in regulating expression of individual host genes involved in human complex traits. Our work also supports the hypothesis that one of the mechanisms by which the microbiome regulates host gene expression is through changes in chromatin accessibility in host cells. Finally, the ability to fine tune the expression of host genes by manipulating the microbiome suggests future therapeutic routes for human wellness.

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P17.30B

Are HDAC potential targets for the epigenetic treatment of pain

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Introduction: Objective of the present study was to evaluate the changes of a subset of histone deacetylases (HDAC) and global DNA methylation level in pain-matrix structures of the brain as the rostroventromedial medulla (RVM), central nucleus of amygdale (CeA) and anterior cingulate cortex (ACC) of formalin test. It involves study of epigenetic agents as potentially therapeutic drugs for management of inflammatory pain.

Methods: All animal studies conformed to the guidelines of IASP regarding investigations. Adult rats receiving intraplantar formalin were tested for antinociception following injection of NSAIDs or/and SAHA in thermal and mechanical tests. The levels of global DNA methylation and HDAC were measured in nuclear extracts of RVM, CeA and ACC neurons. HDAC amount was measured using ELISA kit(Abcam) and global DNA methylation was measured using the Methylated DNA Quantification Kit (Abcam).

Results: Animals exhibited hyperalgesia during formalin test and had high HDAC levels. Treatment with either NSAIDs or 3-day pretreatment with HDAC inhibitors reduce nociceptive mechanical and thermal responses of the formalin test independently. NSAIDs used in combination with SAHA delay the establishment of hyperalgesia and significantly reduce its intensity, also decrease HDAC amount. HDAC and global DNA methylation levels were observed to be different in RVM, CeA and ACC under given conditions.

Conclusions: Our data provide evidence that HDAC inhibitors cause analgesia and are potential targets for epigenetic treatment of pain.

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P17.31C

The discovery of a new role of HDAC8 in skeletal muscle differentiation and in centronuclear myopathy insurgence

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Histone Deacetylase 8 (HDAC8) is a member of class I HDACs with peculiar characteristics: it is subjected to posttranslational phosphorylation and presents differences in substrate specificity, suggesting a unique role for HDAC8 across the HDACs family. For example, HDAC8 activity is specifically inhibited by molecules such as PCI-34051, with a 200-fold higher selectivity than other HDACs. Here, we describe for the first time a specific expression for *HDAC8* during skeletal muscle differentiation. In C2C12 myoblasts the expression of *HDAC8* increases during differentiation and in RD/18 and RD/12 rhabdomyosarcoma cells,

respectively with low and high tumorigenic potential consistent with their differentiating capability, its expression correlates with the differentiation degree. Furthermore, we demonstrate that PCI-34051-mediated-HDAC8-inhibition impairs myogenesis in C2C12 myoblasts and in zebrafish embryos. In zebrafish, when HDAC8 activity is inhibited, only type I fibres are reduced. Moreover, preliminary data show altered expression of *HDAC8* in muscle derived from centronuclear myopathy (CNM) affected patients in comparison with healthy donors. CNM is a condition characterized by skeletal muscles weakness, hypotonia and atrophy mainly in type 1 fibres. Mutations in *MTM1*, *DNM2*, *BIN1*, *RYR1* or *TTN* genes have been associated with CNM, although some patients do not carry mutations in these genes. Hence the need to identify additional genes associated with CNM. In this work, we demonstrate a new role for HDAC8 activity in normal skeletal muscle differentiation, in rhabdomyosarcoma and we suggest a potential involvement of *HDAC8* in CNM insurgence.

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P17.32D

Alterations in linear and back splicing as new players in Huntington's Disease pathogenesis

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Recent findings revealed that *alternative splicing* (AS) might be compromised in Huntington's Disease (HD), a fatal neurodegenerative disorder, caused by 'CAG' trinucleotide repeat expansion in the *Huntingtin* (*HTT*) gene. AS regulation is crucial to the establishment of protein coding isoforms, but also to the biogenesis of circular RNAs (circRNA), stable non-coding RNAs produced by the circularization of exons. The goal of our research was to discover alterations in linear and back-splicing events in *in vivo* and *in vitro* models bearing normal or expanded repeats. To discover alterations in linear splicing, we analyzed genome-wide transcriptomic profiles for striatum, cortex and liver at different developmental time points, quantifying the total number of differential AS events. Our analysis demonstrated that, specifically for the striatum, the total number of differential AS events increased significantly with increasing CAG expansion. Interestingly, only EXON SKIPPING, a specific AS subtype emerged to be strongly and specifically affected. Additionally, we identified the first circRNA originating from the *HTT* locus, expressed in the brain and presenting augmented expression levels with increasing *HTT* CAG size. At genome-wide level, a decreased circRNAs production correlated with mutant HTT expression, revealing 14 circRNAs in neuronal progenitor cells with CAG-length-dependent decreased expression. In conclusion, our results support the idea that AS machinery is responding to HD mutational process altering both linear and back-splicing events locally at the *HTT* locus, but also at the genome-wide level. This knowledge will pave the way to new trials of therapeutic intervention aimed to possibly target spliceosomal-circRNAs alterations.

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P17.33A

Promoter methylation status of interleukin-8 in cystic fibrosis

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Introduction: Cystic Fibrosis (CF) is an inherited disorder characterised by a chronic infection and neutrophil-dominated airway inflammation. This inflammation is characterised by an increased production of pro-inflammatory cytokines in the lung. Interleukin-8 (IL-8), a member of the CXC chemokine family that attracts neutrophils and other leukocytes, has been elevated in Cystic Fibrosis (CF) disease. Epigenomic disregulation has been reported to play a role for the development of chronic inflammation in CF. In the present work we analyzed expression of IL-8 by the CD4+ T-cells from CF patients and DNA methylation status in the promoter region of the IL-8 gene.

Materials and Methods: Genomic DNA from activated CD4+ T-cells of 7 CF patients and 7 controls were purified and subjected to bisulfite modification using EpiTect Fast Bisulfite Kit (Qiagen, USA). Methylation status of the IL-8 gene was determined by methylation-specific polymerase chain reaction (MSP) analysis. PCR products were electrophoresed on 3% agarose gel and visualized. Level of IL-8 in supernatants of activated CD4+ T-cells was measured using ELISA method.

Results: Our results indicate that individuals with CF have a significantly higher percentage of hypomethylation of IL-8 gene promoter regions in CD4+ T-cells compared to healthy controls and expression level of IL-8 was higher than in individuals without CF.

Conclusions: Our results suggest that methylation status of the IL-8 promoter region might be a useful predictor of the complication of Cystic Fibrosis. The study was supported by a grant from the Ministry of Science and Education of Georgia (N59; 2015-30-01).

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P17.34B

Role of intragenic DNA methylation in incomplete penetrance of *IMMP2L*gene microdeletion

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Introduction: Most of CNVs in the human genome exhibit incomplete penetrance with unknown underlined mechanisms. One of such mechanisms may be epigenetic modifications of DNA, in particular, DNA methylation. Here, we report about differential methylation of intragenic CpGs of *IMMP2L* gene in a proband with maternal 7q31.1 microdeletion and de novo 15q11-q13 microdeletion.

Materials and Methods: The proband with Prader-Willi phenotype with signs of autism spectrum disorders was analyzed using aCGH. The presence of 7q31.1 and 15q11-q13 microdeletions in his parents and healthy sibling was investigated by real-time PCR. DNA methylation level in intragenic CpGs of *IMMP2L* gene was measured by bisulfite amplicon next generation sequencing. Genome-wide expression profile was assessed by microarray analysis (Agilent Technologies).

Results: De novo 15q11-q13 microdeletion consistent with Prader-Willi phenotype (OMIM: 176270) as well as 7q31.1 microdeletion of maternal origin were detected in a patient. The 7q31.1 microdeletion affected included only exons 1-2 of the *IMMP2L* gene. *IMMP2L* gene is located in the critical region for the autistic disorder locus on chromosome 7q (AUTS1). The bisulfite sequencing of 87 intragenic CpG-sites revealed the comparable level of methylation in the proband, healthy sibling without microdeletion and father. Whereas a reduced methylation level and increased *IMMP2L* expression were observed in healthy mother's peripheral blood lymphocytes in comparison with a proband.

Conclusion: Obtained results provide evidence for a possible compensation of *IMMP2L* haploinsuffiency in a healthy mother with 7q31.1 microdeletion due to epigenetic mechanisms. This study was supported by Russian Science Foundation, grant 16-15-10229.

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P17.35C

Epigenetic modifications associated with intrauterine growth restriction

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Intrauterine growth restriction is a condition in which the fetus is not able to reach its normal growth potential. The abnormal imprinted gene expression is associated with fetal growth restriction and may be controlled by DNA methylation. DNA hydroxymethylation was recently described and associated with fetal size at birth. The aim of this study was to find biomarkers that allow the prediction of IUGR, studying the effects of imprinted genes (*PHLDA2*, *CDKN1C*, *KCNQ1*, *H19*, *IGF2*, *PEG10* and *MEST*), the effects of KvDMR1 methylation and the global hydroxymethylation.

Quantitative Real-Time PCR, Combined Bisulfite and Restriction Analysis and 5-hmC DNA ELISA were performed in twenty-one term placental samples with IUGR and in nine normal placental samples. Bisulfite sequencing

was performed to confirm KvDMR1 methylation level in five samples.

Gene expression analysis demonstrated a significantly increased expression of *CDKN1C*, *PHLDA2* and *PEG10* genes in IUGR. The results for *CDKN1C* and *PHLDA2* genes are consistent with literature and with parental conflict theory. However, the result for *PEG10* gene suggests a compensatory response to IUGR. The COBRA and BS-sequencing results demonstrated the KvDMR1 hypermethylation in IUGR. The global hydroxymethylation analysis exhibited the presence of 5-hydroxymethylcytosine in the placenta. However, the results showed non-significant changes between the two groups samples.

In this study, *CDKN1C* and *PHLDA2* genes were identified as potential IUGR biomarkers. In addition, the study confirms the presence of 5-hydroxymethylcytosine in placenta and show the KvDMR1 hypermethylation in IUGR samples. The imprinted genes expression increase may result from hypermethylation of KvDMR1, leading to IUGR.

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P17.37A

Analysis of coding and long non-coding RNA expression profiles in Peripheral Blood Mononuclear Cells from Amyotrophic Lateral Sclerosis patients

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Background: Alteration in RNA metabolism, concerning both coding and long non coding RNAs (lncRNAs), may play an important role in Amyotrophic Lateral Sclerosis (ALS) pathogenesis (Gagliardi et al., 2018). We have analyzed the regulation of mRNAs and lncRNAs in Sporadic (SALS) and mutated ALS patients and in matched controls in peripheral blood mononuclear cells (PBMCs) and we have correlated the founded RNA regulation with ALS onset and disease progression.

Materials and Methods: RNAs from PBMCs of SALS, SOD1, FUS, TARDBP mutated patients and matched controls have been used for RNA-seq experiments (TruSeq Stranded RNA Library Prep, Illumina) that have been analyzed by R package EBSeq. RNA-seq data has been validated by Real time PCR.

Results: In SALS patients, a total of 775 deregulated mRNAs (45% down and 55% up) and 235 lncRNAs (76 % down and 24% up) were found. 84 of lncRNAs are reported as antisense, 91 as lincRNAs, while remaining 60 RNAs classified as processed transcripts or intronic sense RNAs. Interesting, the analysis of SALS patients classified by fat and slow progression (Gomeni et al., 2013) showed a most important deregulation and the involvement of different pathways in faster-progressing patients. About mutated patients, the most interesting data are about FUS patients that showed the major number of deregulated genes.

Conclusions: This investigation has confirmed the importance of extending our knowledge on molecular alterations of transcriptome in ALS disease offering numerous starting points for new investigations about pathogenic mechanism involved in ALS disease.Grants: ALS-ARiSLA, FRRB 2015-0023

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P17.38B

Peripheral blood DNA methylation as potential biomarker of malignant pleural mesothelioma in asbestos-exposed subjects

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Introduction: Malignant pleural mesothelioma (MPM) is an aggressive tumour strongly associated with asbestos exposure. MPM prognosis is poor, highlighting the need for non-invasive tests to monitor asbestos-exposed people aiming at an early MPM diagnosis.

Methods: We investigated genome-wide DNA methylation (HumanMethylation450-BeadChip) in 163 MPM cases/137 cancer-free controls (82/68 Training-Set; replication 81/69 Test-Set), all of them with asbestos exposure assessment.

Results: We found case/controls differential methylation (>800 CpG sites, $_{Pfdr}$<0.05) mainly in immune system related genes, and accelerated methylation age of white blood cells in MPM cases (extrinsic epigenetic age acceleration, $p = 0.009$). Considering the "top" differentially methylated signals, 7 single-CpGs and 5 genomic regions replicated with similar effect size in the Test Set (p_{fdr}<0.05). The top hypomethylated single-CpG (cases *vs* controls effect size<-0.15, p_{fdr} <0.05 in both Training and Test sets) was detected in *FOXK1* (Forkhead-box K1) gene, an interactor of BAP1 which was found mutated in MPM tissue and as germline mutation in familial MPM. In the Test set, comparison of receiver operating characteristic (ROC) curves analysis of two models, including/excluding methylation, showed a significant increase in case/control discrimination when considering DNA methylation together with asbestos exposure (AUC=0.81 *vs* AUC=0.89, DeLong's test $p = 0.0013$). Additionally, preliminary results from mediation analysis suggest asbestos-related differential methylation.

Conclusions: We identified signatures of differential methylation in DNA from whole blood between asbestos exposed MPM cases and controls. Our results suggest the potential use of DNA methylation profiles in blood to develop non-invasive tests for MPM detection in asbestos-exposed subjects.

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P17.39C

Metformin-induced alterations of transcriptome profile in healthy individuals

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Introduction: Metformin is the first-line antidiabetic agent used in pharmacotherapy of type 2 diabetes to improve glucose homeostasis. Nevertheless, additional therapeutic directions such as cancer prevention, treatment of polycystic ovary syndrome and neurodegenerative diseases have been highlighted lately justifying the pleiotropic effect of the drug. Despite the identification of AMPK as the major mediator of its effects, exact mechanisms of metformin action remain obscure.

Materials and Methods: The longitudinal study enrolled 25 healthy volunteers of European descent, receiving oral 850mg dose of metformin twice-daily for seven days. RNA was isolated from whole blood samples, collected at three consecutive time points: before metformin administration, 10 hours after the first dose and at the end of metformin treatment. RNA-seq was performed on Ion Proton™ System and Ion PI™ Chip. For bioinformatic analysis Trimmomatic 0.36, STAR 2.5.3a, edgeR and DAVID 6.8. tools were applied.

Results: In total 681 differentially expressed genes were identified (FDR<0.05), among them genes related to inflammatory responses, promoting enrichment of intestinal immune network for IgA production and cytokine-cytokine receptor interaction pathways. Four additional functional gene clusters were revealed after consideration of subject-specific effects including ribosomal genes, snoRNAs and genes relevant to insulin production (HNF1B, HNF1A, HNF4A, GCK, INS, NEUROD1, PAX4, PDX1, ABCC8, KCNJ11) and cholesterol homeostasis (APOB, LDLR, PCSK9).

Conclusion: In healthy individuals universal metformin-induced alterations of global gene expression profiles in white blood cells are associated with immune responses, while subject-specific effects, which tendency to be more permanent are related to energy metabolism. Funded by ERDF Project Nr.1.1.1.1/16/A/091.

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P17.40D

Correlation status of BRAF V600E mutation and miR-137 and miR-181b expression in Iranian PTC patients

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Introduction: Papillary thyroid carcinoma (PTC) as well-differentiated thyroid carcinoma is the most frequent endocrine malignancy. Abnormal expression of micro-RNAs (miRs) is related to numerous carcinomas and malignancy. We planned to assess the miR-137 and -181b expressions and their association with BRAF V600E mutation and clinicopathological features in Iranian PTC cases.

Materials and Methods: In total, 90 primary thyroid samples (60 PTC and 30 benign with multinodular goiter (MNG)) and two cell lines including human PTC (B-CPAP) and human embryonic kidney (HEK293) were surveyed. All clinicopathological information of patients was obtained and a pathologist confirmed specimens. T1799A mutation of exon 15 in the BRAF gene was identified. MiRs expression was assessed by quantitative reverse transcriptase real-time PCR.

Results: Of 60 PTC cases and 30 MNG subjects, 75 and 83.3 % were female, respectively. MiR-137 and -181b were upregulated in PTC compared to MNG (P=0.010 and 0.007, respectively). Their levels were upregulated in B-CPAP compared to HEK293 cell line (P<0.001). Moreover, these two miRs were upregulated in PTC patients with BRAF V600E mutation (P=0.036 and 0.002, respectively), tumor size ≥2cm (P=0.002 and 0.010, respectively), extracapsular invasion (P=0.034 and 0.027, respectively), lymphovascular invasion (P=0.038 and 0.032, respectively). The miR-181b expression was elevated in patients with multifocal tumors (P=0.045) and the miR-137 level was increased in PTC cases with lymph node metastasis (P=0.011).

Conclusions: Upregulation of miR-137 and -181b have been confirmed in PTC tumors and cell line. These two miRs associated with BRAF V600E mutation and malignancy of PTC.

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P17.41A
Identifying eQTL influence on gene expression through microRNAs

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Introduction: microRNAs drive coordinated expressional changes of their target genes and trigger functional shift in cells. Placental microRNAs are specifically involved in trophoblast differentiation and function. Additionally, miRNAs transferred by extracellular vesicles released from trophoblastic cells exhibit critical immunomodulatory role at the maternal-fetal interphase. Single nucleotide variants (SNVs) associated with the expression level of genes are defined as expression quantitative trait loci (eQTLs). The aim of my PhD project is to identify placental eQTLs modulating the expression of microRNAs and to understand their downstream effect on the placental transcriptome.

Materials and Methods: Placental miRSeq (unpubl. data) and genotyping (Kasak et al 2015) datasets were subjected to genetic association testing for eQTL discovery, implicated in PLINK v1.07 (Purcell et al 2007). microRNA target genes were predicted using the BCmicrO database (Yue et al 2012). Association testing between identified miRNA eQTLs and placental expression levels of predicted target genes were tested in PLINK. Correlations between the expression profile of placental miRNAs (miRSeq dataset) and transcripts (RNA-Seq dataset; Sõber et al 2015) were analyzed using DESeq2 platform (Love et al 2014).

Results: In total, 11 placental microRNAs were detected that were expressionally modulated by SNVs. Research was focused to six miRNAs (hsa-miR-130b, hsa-miR-490-3p, hsa-miR-3927, hsa-miR-941, hsa-miR-301b, hsa-miR-152) that have been implicated in the placental function and/or pregnancy complications. For each prioritized miRNA several novel target genes and involved biological pathways were identified.

Conclusions: miRNA eQTLs may represent additional modulators of the placental transcriptome, placental function and pregnancy course.

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P17.42B
Tool for pathogenesis analysis of nucleotide variants based on high-confidence human miRNA-mRNA interactions

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miRNAs play a key role in the regulation of gene expression, while a majority of miRNA-mRNA interactions remain unidentified. Different miRNA prediction programmes have a lack of consensus about predicted miRNA binding sites. Crosslinking immunoprecipitation (CLIP)-seq experimental techniques allow revealing transcriptome-wide binding sites of RBPs. Today there are many data of CLIP-seq experiments with AGO2 protein which allow to use it for high-throughput characteristics of miRNA-mRNA interactome of human.

We collected results of 79 AGO2-CLIP-seq data (18 PAR-CLIP and 61 HITS-CLIP) from 11 studies in 9 cell types. We also took data from two modified CLIP-seq studies (CLASH, CLEAR-CLIP) that straightforwardly detect miRNA–mRNA pairs as chimeric reads.

Totally, we identified 156 thousand miRNA binding regions and formed a subset of 45 thousand high-confidence regions that were identified in at least two different experiments. The analysis of high-confidence miRNA binding sites revealed tissue-specific interactions for two predominate cells: HEK293 and Huh7.5. On the other hand, we obtained a group of 13,6 thousand "house-keeping" miRNA binding regions that were identified in predominant cells.

For analyzing nucleotide variants from patients with an inherited disease which could be caused by breaking in the miRNA-mRNA interactions, we developed a tool based on the high-confidence regions.

We identified the subset of 45 thousand high-confident human miRNA binding regions that were arranged in the tool. Hence, it will be a valuable resource that should provide additional insights into the identification new molecular mechanisms of hereditary diseases caused by breaking in the miRNA-mRNA interactions.

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P17.43C

mRNA/microRNAin extracellular vesicles from ALS and AD patients: a specific signature for Neurodegenerative Diseases

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Introduction: Exploring robust biomarkers is essential for early diagnosis of neurodegenerative diseases. Blood contains microvesicles (MVs) and exosomes (EXOs), extracellular vesicles of different sizes and biological functions, which transfer mRNA, miRNAs, or proteins among different cell types. Aim of our study was to investigate mRNA/miRNA signature in plasma derived MVs and EXOs of Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's Disease (AD) patients.

Materials and Methods: MVs and EXOs were isolated from plasma of 5 sALS, 5 AD patients and 5 healthy volunteers (CTR) by ultracentrifugation and whole RNA was extracted. mRNA libraries were prepared by TruSeq Stranded Total RNA kit (Illumina) (60 million reads). miRNA libraries were prepared by TruSeq Small RNA Library kit (Illumina) (5-8 millionreads). Data were analyzed with ad hoc Bioconductor packages.

Results: RNA-seq analysis showed different profiles between ALS, AD and CTR. In EXOs, we have detected 8 deregulated genes (DE) in ALS patients and 72 in AD group. In MVs, we found 17 DE in ALS, 173 DE genes in AD. In AD patients, 3 DE miRNA were detected in EXOs, while 65 miRNA DE in MVs. In ALS group 5 miRNA were DE in exosomes while 9 were altered in MVs.

Conclusions: About ALS, the most interesting data concern the exosome coding transcriptome where we identified 8 coding RNAs. Regarding AD, a distinct miRNA signature (65 miRNA) was identified in microvesicles. Our results hypothesized a mRNA/miRNA signature in plasma derived EVs that may be an specific biomarker for neurodegenerative diseases.

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P17.44D

Genomic methylation and mtDNA copy number variation in atherosclerosis

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Introduction: Mitochondrial DNA is controlled by nuclear genes, and there is increasing evidence that epigenetic

mechanisms play significant role in mtDNA function and maintenance. The aim of the study was to explore epigenetic links between nucleus and mitochondrion in atherosclerosis.

Materials and Methods: We studied mtDNA copy number (by real time PCR), mtDNA D-loop methylation (by bisulfite treatment, PCR and Illumina sequencing) and LINE-1 methylation (by pyrosequencing) in blood and carotid atherosclerotic plaques of patients subjected to endarterectomy (N=50).

Results: We registered very low level of mtDNA methylation in the D-loop (0.5%-2%, depending on position), both in CpG sites and for non-CpG cytosines. However, rate of A and G calls in the reference C positions, and C calls in the reference T positions (indicating PCR/sequencing error rate) was even less (about 0.3%). Blood samples and plaques did not differ by mtDNA methylation level. Median value of mtDNA copy number per cell was higher in plaques than in blood cells. There was negative correlation of mtDNA copy number with LINE-1 methylation level in plaques (Spearman r=-0.54), but not in blood.

Conclusions: The results indicate that there might be some epigenetic control of mtDNA replication in the atherosclerotic lesions. Taking into account that decreased overall genomic methylation is considered as negative factor, the increased mtDNA copy number in atherosclerotic plaques and its negative correlation with LINE-1 methylation can be explained by compensatory amplification in response to mitochondrial dysfunction and oxidative stress in the affected vessels.

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P17.45A

Molecular diagnosis and genetic bases of multilocus methylation defects in Beckwith-Wiedemann and Silver-Russell syndromes

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Introduction: Several patients with imprinting disorders (IDs) exhibit epigenetic alterations not only at the disease-specific loci but extended to other imprinted genes, the so called multilocus methylation imprinting disturbance (MLID). Causative mutations in genes involved in methylation establishment have been identified only in few cases and in the majority of MLID cases the causative defect remains unknown.

Materials and Methods: We developed a quantitative methylation test by MassARRAY to detect alterations at 12 imprinted regions in 21 pre-and post-natal Beckwith-Wiedemann (BWS) and 7 post-natal Silver-Russell (SRS) patients. Targeted NGS analysis was performed to identify possible causative mutations in a panel of 25 genes involved in methylation establishment and maintenance.

Results: About 50% of BWS and 29% of SRS patients showed MLID, with loss of methylation confined to maternally imprinted loci. NGS allowed the detection of two novel mutations in *NLRP2* and *ZFP42* genes in two MLID patients. In our cohort, MLID appears higher frequent in females, with a male-to-female ratio of 1:4.

No significant differences in clinical features between single- and multilocus patients were observed. However, a number of clinical features appeared more frequent in BWS MLID patients, i.e. macroglossia, outer ears anomalies and *facial nevus flammeus*.

Conclusions: MLID appeared a frequent finding in BWS/SRS patients. The hypomethylation confined to maternally imprinted loci, suggests alterations in *trans-acting* factors involved in methylation establishment/maintenance in the oocyte, as confirmed by mutations found in MLID patients. *In silico* analysis and bioinformatics modelling confirm the pathogenic effects of these mutations.

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P17.46B

Do maternal effect mutations in NLRP5 cause ICR1 hypomethylation in Silver-Russell syndrome patients?

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Imprinting disorders comprise a group of clinical entities sharing parent-specific changes in the epigenetic signature of specific genomic loci. The imprinting disorder Silver-Russell syndrome (SRS) is caused by loss of methylation (LOM) of the imprinting center region 1 (ICR1) on chromosome 11p15.5 in 30-60% of patients. In nearly 10% of these patients, aberrant methylation on additional chromosomes have been detected, establishing a spectrum of multilocus imprinting disorders (MLIDs). By investigating different tissues, MLID cases in the ICR1 LOM cohort increases up to 38%. Maternally-provided factors in the ooplasm are postulated for the establishment and maintenance of imprinting marks in the embryo, leading to MLIDs by so-called maternal effect mutations. Several of these maternal effect genes are members of the *NLRP* gene family[1].

This study questions whether maternal effect mutations are also cause of apparently isolated ICR1 LOM. We analysed peripheral blood lymphocytes from 21 mothers of ICR1 LOM patients by a targeted next-generation sequencing approach (*NLRP2-14, TRIM28, KHDC3L*). Heterozygosity for variants in *NLRP5* were identified in two mothers: NM_153447.4:c.68T>A (p.(Val23Asp); rs753824534) and NM_153447.4:c.3259G>A (p.(Glu1087Lys), both listed as rare/pathogenic, compatible with maternal-effect mutations. It has to be discussed whether our findings are in line with an association of isolated ICR1 LOM and maternal effect mutations in *NLRP5* or whether MLID remained undetected in the children of these two mothers. Thus, identification of further factors causing ICR1 LOM and studies of additional tissues with more sensitive approaches should help distinguishing between both possibilities.

1. Monk, D. et al. *Reproduction* **154**, R161-R170 (2017).

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P17.47C

Evaluation of Serum miRNA (mir-26a, mir-29a, mir-133a) Expression Levels in Patients with Osteogenesis Imperfecta

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Objective: Osteogenesis Imperfecta(OI) is associated with long bone deformities and fractures, accompanied by a genetic connective tissue disorder. According to their clinical, inherited, and radiological characteristics 17different types of OI has been reported. There are autosomal dominant(OD) and autosomal recessive(OR) transmission, although inheritance patterns differ according to types. MicroRNAs(miRNAs) play important roles in processes such as ossification, osteoporosis, osteoblastic proliferation, differentiation. In this study we aimed to identify the role of microRNAs in the clinical heterogenity of OI, contribute to the understanding of their utility as biomarker,and also to determine the expression differences of bone-associated miRNAs(miR-26a,miR29a,miR-133a) between the patients and healthy controls in order to search for their possible use as a new therapeutic target.

Method: 0-18 years age matched 26 OI patients that followed by pediatric genetics, endocrinology and physical rehabilitation clinic and 16 healty control group were involved. MiRNeasy Serum / Plasma Kit (Qiagen, 217184) was used for the isolation of miRNA(mir-26a,mir-29a,mir-133a) from the plasma. The values determined by quantitativePCR were normalized using REST programme.

Results: There was a significant difference between the miRNA levels ofthe patient and control groups and the miRNA levels of the patient group werehigher. Serum calcium and vitamin D levels of patients with higher mir133alevels were lower than those of the other groups.

Conclusion: OI has no definitive treatment. Identification of some biomarkers may be important in the detection of the disease at an early stage, monitoring the response to treatment, and determining miRNAs that are likely to be a new therapeutic targets.

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P17.48D

Integrated DNA methylation analysis identifies topographical and tumoral biomarkers in pilocytic astrocytomas

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Introduction: Pilocytic astrocytoma (PA) is the most common pediatric brain tumor. Although genetic and epigenetic alterations characterizing PA from different localizations have been reported, the role of methylome alterations in PA development is still not clear. In order to investigate whether distinctive methylation patterns may define biological relevant subgroups of PAs, we characterized the DNA methylation profiles of 20 tumors and 4 non-tumoral brain samples.

Materials and Methods: genome-wide DNA methylation analysis was performed using Illumina Infinium HumanMethylation27K BeadChips. Gene expression levels of selected differentially methylated genes, evaluated by qRT-PCR, were compared to commercially available human normal brain samples and to GTEx expression data observed in different normal brain sites.

Results: we identified distinct methylation profiles characterizing PA from different locations (supratentorial *vs* infratentorial) and tumors with onset before and after 3 years of age. Gene expression analysis of *TOX2* and *IRX2*, hypermethylated in supratentorial PA, revealed a decreased expression in supratentorial compared to infratentorial PAs. The expression level of *IRX2* in the tumor samples resulted in line with that observed in the normal brain sites. In contrast, *TOX2* showed an opposite expression pattern in PA compared to that in normal brain sites.

Conclusions: our study revealed brain-region and age-related specific methylation patterns in PA and identified *IRX2* as a topographic biomarker and *TOX2* as a promising tumoral biomarker.

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P17.50B
Epigenetic signature of preterm birth in adult twins

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Preterm birth is a leading cause of perinatal mortality and long-term health consequences. Epigenetic mechanisms may have been at play in preterm birth survivors and these could be persistent and detrimental to health later in life. We performed a genome-wide DNA methylation profiling adult twins of premature birth to identify genomic regions under differential epigenetic regulation in 144 twins with a median age of 33 years (age range: 30-36). Association analysis detected three genomic regions annotated to the *SDHAP3, TAGLN3*, and *GSTT1* genes on chromosomes 5, 3, and 22 (FWER: 0.01, 0.02 and 0.04) respectively. These genes display strong involvement in neurodevelopmental disorders,cancer susceptibility and premature delivery. The three identified significant regions were successfully replicated in an independent sample of twins of even older age (median age 66, range: 56-80) with similar regulatory patterns and nominal p values <5.05e-04. Biological pathway analysis detected 5 significantly enriched pathways all explicitly involved in immune responses. The study provides novel epigenetic evidence for the association between important genes/pathways and premature delivery and meanwhile reveals that preterm birth, as an early life event, could be related to differential methylation regulation patterns observable in adults and even at high ages which could potentially mediate susceptibility to age-related diseases and adult health.

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P17.51C
The effect of TP63 expression on the methylation level of CpG-sites located in enhancers of the genes associated with epithelial-mesenchymal transition in prostate cancer

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Introduction: Aggressive forms of prostate cancer (PCa) are often associated with epithelial-mesenchymal transition (EMT). Thus, the study of the master regulators of epithelial fate (TP63) can help to understand the potential mechanisms of formation non-indolent forms of PCa.

Materials and Methods: Whole-genome methylation, RNA-sequencing, and copy number variation data of patients with PCa from The Cancer Genome Atlas (TCGA) portal were used for this research. The regulatory networks were reconstructed from EWAS (epigenome-wide association study) based on mixed linear models. ChIP-seq data were downloaded from GEO.

Results: We obtained a cluster of genes and CpG-sites downregulated in PCa samples. Further, we found the association between the cluster and the EMT in prostate cell lines (data from Olsen et al. 2013). The master regulator of this cluster was TP63 because promoters were significantly enriched with TP63 binding sites (p-value=5e-26), and TP63-knockdown altered expression of cluster genes. The expression of cluster genes was highly correlated (Spearman < -0.8) with methylation level of 556 CpG-sites located in the enhancers and super-enhancers (eCpG-sites, significant enrichment with H3K27ac and H3K4me peaks). This eCpG-sites interacted with promoters of the genes associated with EMT.

Conclusions: We found the cluster of genes and CpG-sites that associated with EMT and were regulated by expression of TP63. Expression of cluster genes highly correlated with eCpG-sites. We speculate that expression of TP63 effects methylation level of eCpG-sites because TP63 is a pioneer transcription factor. This work was supported by the Russian Foundation for Basic Research (project No. 17-29-06063).

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P17.52D

First international consensus statement on diagnosis and management of pseudohypoparathyroidism and related disorders

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Introduction: Pseudohypoparathyroidism (PHP) and related disorders lead to a wide spectrum of abnormal physical characteristics, neurocognitive and endocrine abnormalities that share a common PTH/PTHrP signaling pathway. The clinical and molecular overlap of these disorders leads to difficulties in clinical and molecular diagnosis which prompt to the possibility of incorrect management of these patients. Over the past 30 years, incredible progress has been made on the pathophysiology of these disorders by physicians and research networks. However, caregivers and patients are lacking guidelines for the daily life management of patients. Our aim was to help them from the clinical diagnosis, to the molecular confirmation up to the management of the most frequent manifestations of these rare diseases.

Methods: A consensus statement was prepared for 2 years to produce recommendations for clinical and molecular diagnosis and management of patients with PHP and related disorders. The approach comprised 2 pre-consensus meetings, an expert consensus meeting, and a Delphi-like methodology, adjusted to rare diseases.

Results: After literature search using PubMed, >800 papers published since 1990 to 2016 have been reviewed and recommendations on clinical and diagnosis and management on PHP and related disorders have been voted and approved with different levels of evidence: 14 recommendations on clinical diagnosis, 11 on molecular diagnosis and 39 on management and treatment.

Conclusion: Overall, a coordinated approach from infancy through adulthood should help us to improve the care of patients affected by these disorders.

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P17.53A

Functional validation of an epigenomic variant associated to retinitis pigmentosa

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Introduction: Recently, an intronic variant in *OFD1* (chrX:13768358 A>G), which decreases the amount of properly spliced *OFD1* transcript, was reported in an

individual with a subtype of non-syndromic retinal degeneration (RD) called retinitis pigmentosa 23 (RP23). Our lab has identified an individual with a similar X-linked phenotype who has a novel intergenic variant within a DNase I hypersensitive site (DHS) upstream of *OFD1*. We hypothesize that this variant is affecting expression levels of *OFD1*.

Materials and Methods: *OFD1* mRNA and protein expression levels of the proband relative to male controls were measured in primary fibroblast cell lines using droplet digital PCR (ddPCR) and immunofluorescence. 4C-seq was also used to capture physical interactions between the DHS and *OFD1* in an adult retinal pigment epithelial cell line (RPE1).

Results: Preliminary ddPCR results suggest that the proband expresses lower levels of *OFD1* than controls. However, 4C-seq results suggest that the DHS does not interact with the *OFD1* promoter in adult RPE. Therefore, further functional validation is required to determine the specificity of our variant to *OFD1* expression and to determine how and at which developmental stage our variant alters the activity of the DHS.

Conclusions: Although preliminary results suggest that the proband expresses lower levels of *OFD1*, the mechanism by which disruption of the DHS causes disease is inconclusive. Additional functional assays will elucidate the regulatory and functional consequences of our variant, and will provide insight about the ways epigenetics and regulatory genetic mechanisms can influence RD.

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P17.54B

DNA methylation and gene expression profiling of CD4+ T cells in rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is an autoimmune disease causing chronic inflammation preferentially in joints. Altered function in CD4+ T cells has been well documented for its association with the development of RA. In this study, we investigated RA-specific gene expression and DNA methylation in CD4+ T cells.

Materials and Methods: The CD4+ T cells from 82 Korean patients with RA and 40 healthy controls were examined by using the Illumina HT-12 expression and Infinium methylation 450K beadchips, respectively. After a series of standard quality control and normalization procedures, differentially expressed genes (DEGs) and differentially methylated positions (DMPs) were assessed using a linear model adjusting for the slide batch effect, age, sex and/or relative cell composition.

Results: We identified 55 DEGs and 91,067 DMPs at the 5% FDR threshold (e.g., $P=1.61\text{x}10^{-5}$ for *ASCL2* in the DEG analysis; $P=9.69\text{x}10^{-4}$ for cg11373604 in the DMP analysis). In a joint analysis for DEGs and DMPs, higher expression levels in 15 DEGs were explained by lowered DNA methylation at neighboring DMPs ($P<0.05$ for a DEG-DMP correlation). For example, the expression level of *ASCL2* and the methylation level at cg11373604 represented a strong negative correlation ($P=8.27\text{x}10^{-6}$; $r=-0.424$).

Conclusions: This study revealed a number of RA-specific expression and DNA methylation in an RA-relevant immune cell type, CD4+ T cells, and identified plausible RA-specific DMPs that lead expressional variances in DEGs.

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P17.55C

Genome-wide analysis of RNA editing levels in human blood identified interactions with mRNA processing genes and suggested correlations with biological and drug-related variables

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A-to-I RNA editing is a post-transcriptional modification catalyzed by ADAR enzymes, that deaminate Adenosines (A) into Inosines (I). Most of known editing events are located within inverted ALU repeats, but they also occur in coding sequences and may alter the function of encoded proteins. RNA editing contributes to generate transcriptomic diversity and it is found altered in cancer, autoimmune and neurological disorders. However, little is known about how editing process could be influenced by genetic variations, biological and environmental variables. We analyzed the dynamic of RNA editing in human blood using RNA-seq data from 459 healthy individuals and

obtained detectable editing levels for 2,079 sites. Analysis of gene expression showed that in blood ADAR expression accounts for ~13% of observed variability in overall editing level. After removing ADAR effect, we found significant associations for 1,122 genes, mainly involved in RNA processing and identified 276 potential ADARs interactors, including 9 ADARs direct partners. In addition, association analysis between editing levels principal components and 35 biological and drugs intake variables revealed 24 factors potentially influencing RNA editing in blood, including sex, age, BMI, drugs and medications. Finally, we identified genetic loci associated to global editing levels, including known ADAR eQTLs and a small region on chromosome 7, containing LOC730338 lincRNA gene. Our data provides a detailed picture of RNA editing and its variability in human blood, giving interesting insights on the mechanisms behind this post-transcriptional modification and genetic and environmental factors involved in its regulation.

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P17.56D

Histone acetylation is reduced in Rubinstein-Taybi patients: epigenetics role in the syndrome

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Introduction: Rubinstein-Taybi (RTS) is a rare autosomal-dominant disorder characterized by growth retardation, cognitive impairment, facial abnormalities and high incidence of neoplasia. CREBBP mutations account for 50-60% of RTS cases. CBP, encoded by CREBBP, is a transcriptional co-activator with lysine acetyltransferase (KAT) activity, targeting specific positions on histones H2B (H2BK15) and H3 (H3K18 and H3K25). The aim of this study was to assess the relationship of CREBBP mutations and impaired KAT activity of CBP in RTS patients.

Materials and Methods: Histones were purified from nuclear blood cells of 18 RTS patients with a representative variety of CREBBP mutations, and 6 age-matched controls. Percentage of acetylation (Ac%) of specific positions H3K18 and H3K23 was determined by ELISA. Additionally, quantification of H2B15KAc relative to total H2B was carried out by Western Blot in 6 RTS and 4 controls.

Results: RTS patients showed a 35- 40% of the control acetylation level on H3K18 and H3K25, which implied a statistically significant reduction compared to controls. More than 70% of the RTS patients showed a consistent Ac% reduction in the two specific H3 acetylation sites to half of the control level. Moreover, levels of H2B15K were found to be significantly lower in RTS patients than in controls.

Conclusions: A wide spectrum of CREBBP mutations in RTS patients was associated with a general deficit in specific histone acetylation by CBP, providing additional understanding of the molecular etiology of the syndrome. These preliminary findings may also extend epigenetics as a key factor and potential therapeutic target in RTS.

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P17.57A

Post-transcriptional regulation of *RUNX2* gene during evolution: implications in the ossification process in nonsyndromic craniosynostosis

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Runt related transcription factor 2 (*RUNX2*) encodes the master transcription factor in skeletal development. *RUNX2* affects skull ossification and is thought to be involved in the progressive skull "globularization" of anatomically-modern humans (AMHs) compared with extinct species. *RUNX2* mutations cause disorders of skull development, including craniosynostosis. The aim of our study was to comparatively characterize the genomic structure of *RUNX2* in AMH and extinct hominins, and to infer putative functional correlations for human skull morphogenesis, using non-syndromic craniosynostosis (NCS) as a disease model. *RUNX2* counts 11 splice variants, alternatively transcribed from two promoters, and featuring two alternative 3'UTRs. *In silico* analysis allowed detecting all nt changes between AMH and Neanderthal/Denisova, within noncoding regulatory sequences and predicting the effect of changes in the 3'UTRs on miRNA binding. miRNA and *RUNX2* splice isoforms were analyzed by qPCR, during the *in vitro* osteogenic differentiation of suture-derived cells. We found that miRNA expression was modulated during the ossification process and correlated with *RUNX2* expression. Particularly the isoform containing the second 3'UTR was apparently stabilized by miRNA binding. *In silico* protein modeling showed the presence of an additional DNA-binding leucine-zipper domain which is lacking in the isoform with the first 3'UTR. Our data suggest that *RUNX2* genomic evolution may have affected miRNA binding leading to changes in epigenetic regulation of the gene, mostly affecting a specific splice isoform. This isoform is apparently able to bind the target DNA with increased

affinity, thus influencing the expression of bone-specific genes plausibly involved in "globularization".

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P17.58B

A CTCF- dependent chromatin interaction ensures robust enhancer - promoter communication at the *Shh* locus

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Sonic Hedgehog (*Shh*) is expressed in the distal-posterior part of developing limb buds and controls digit growth, number and identity. The ZRS enhancer, which regulates *Shh* transcription in developing limbs, is located in the intron 5 of the constitutively transcribed gene *Lmbr1* and has been involved in numerous patient cases with limb malformations. *Shh* and the ZRS communicate through a 1Mb-large stable chromatin interaction. However the mechanism facilitating this long-range contact is not known yet. Using a series of CRISPR/Cas9 engineered alleles and 4C-seq experiments in mouse embryos, we aim to elucidate the role of the *Lmbr1* constitutive transcription as well as CTCF in the establishment of the *Shh*-ZRS chromatin interaction. Deletion of the *Lmbr1* promoter abolishes the transcription over the ZRS, yet 4C-seq experiments showed that the contact frequency between *Shh* and its enhancer remains unchanged in these embryos allowing for normal *Shh* expression. In contrast, the removal of CTCF binding sites around and within the ZRS results in the emergence of compensatory CTCF binding sites as well as in altered 3D chromatin architecture, diminished *Shh* transcription and skeletal abnormalities. Our results suggest that CTCF acts to support a robust and permissive enhancer-promoter interaction that ensures normal *Shh* expression. This CTCF-dependent regulatory mechanism provides a framework to understand the pathomechanism of unsolved structural variants described at the *Shh* locus in patients with skeletal abnormalities.

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P17.60D

An inflammation-related SNP in XPO1 5'UTR regulates protein amount by altering m6A mRNA methylation

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XPO1, located in the Immunochip region 2p16.1, mediates the nuclear export of other proteins and some RNAs. The SNP rs3087898, in the 5'UTR of the XPO1 transcript, has been associated to celiac disease (CeD), an immune disorder of the small intestine. N'6 methyladenosine (m6A) is the most frequent methylation mark in mRNAs and lncRNAs, affecting several aspects of RNA metabolism. Interestingly, XPO1 shows m6A methylation signals in the 5'UTR, very near the associated SNP, which could influence the function of the protein. Thus, we wanted to investigate whether the CeD-associated SNP could affect the methylation levels of XPO1 mRNA and subsequently, its protein levels. m6A immunoprecipitation in intestinal cells confirmed the presence of mRNA methylation on the 5' UTR of XPO1. Moreover, cells heterozygous for the rs3087898 SNP showed preferential methylation in the CeD-associated allele. After treatment with cycloleucine, an m6A inhibitor, both XPO1 mRNA and protein amounts tend to decrease, suggesting that there will be lower levels of XPO1 in the individuals with the protection genotype. Concordantly, when we quantified the amounts of XPO1 in intestinal biopsies from individuals with different genotypes, we observed that XPO1 levels were higher in the presence of the risk allele. These data suggest that the SNP rs3087898, associated with CeD, influences the methylation levels of XPO1 mRNA and regulates XPO1 protein translation, which could in turn affect the nuclear export of certain proteins that have been related with disease development as STAT3 or IKBa. Funding: ACM; PI2018007 Basque Government; ISCIII PI13/0120-PI16/0258; CIBERDEM

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P17.61A

miR-146a single nucleotide polymorphism rs2910164 (G>C) regulates miR-146a expression in osteoarthritic chondrocytes

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Introduction: Deregulation of microRNAs is involved in osteoarthritis (OA) pathogenesis. MiR-146a contributes to inflammation and cartilage degradation observed in OA joints. Single polynucleotide polymorphisms in miRNAs genes may influence miRNAs expression and have often been associated with diseases. In our study, we investigated whether miR-146a rs2910164 (G>C) predisposes to OA susceptibility and its possible functional role to miR-146a expression in osteoarthritic chondrocytes.

Material and Methods: Genetic association analysis was performed using a cohort of 900 Greek OA patients and 750 healthy controls. Genomic DNA was extracted from blood and genotyped using PCR-RFLP. Articular cartilage was obtained from 25 patients with primary osteoarthritis undergoing total knee replacement surgery and 15 healthy individuals with no history of joint disease. Total RNA was extracted from cultured chondrocytes and expression levels of miR-146a were evaluated using real-time PCR.

Results: miR-146a rs2910164-GC and CC genotypes were not associated with an elevated risk of OA compared to GG genotype. Moreover, we observed that miR-146a expression levels were significantly decreased in osteoarthritic chondrocytes compared to normal and that the relative expression levels of miR-146a in chondrocytes of OA patients carrying the rs2910164-GC genotype were significantly lower than that ones with GG genotype.

Conclusion: Our study demonstrates, for the first time, that although miR-146a rs2910164 (G>C) is not a susceptibility factor for OA, miR-146a rs2910164-GC genotype is associated with reduced miR-146a expression levels in osteoarthritic chondrocytes. These data provide strong evidence that genetic variations could regulate the expression levels of microRNAs that are linked to OA pathogenesis.

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P17.62B
Differential expression of long non-coding RNAs in distinct histological type of thyroid cancer

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Introduction: Long non-coding RNAs (lncRNAs) are assumed to participate in cancer pathogenesis and can be considered as potential markers or therapeutic targets. Investigations of lncRNA in thyroid cancer are not sufficient and limited mostly to papillary thyroid cancer.

Materials and Methods: 9 datasets assessed with Affymetrix Human Genome U133 Plus 2.0 Array and published in GEO were selected. Samples with known radiation exposure were excluded. The analyzed set included normal thyroid tissue (106 samples); tumor tissue of classic papillary carcinoma (67 samples); follicular variant of papillary carcinoma (35 samples); poorly differentiated carcinoma (18 samples); anaplastic carcinoma (45 samples). Datasets of RNAseq from SRA and TCGA repositories were selected for validation. Differential expression of 4398 non-coding genes (including 1982 lincRNA and 1518 antisense RNA) was assessed. Genes with FDR adjusted p-value ≤ 0.01 and Fold Change > 2 were considered to be differentially expressed.

Results: In classic PTC 140 lncRNAs were found to be differentially expressed (including 54 lincRNAs and 44 antisense RNAs). In follicular PTC differential expression was revealed for 88 lncRNAs (including 36 lincRNAs and 29 antisense RNAs). In poorly differentiated thyroid carcinomas 146 lncRNAs were differentially expressed (including 62 lincRNAs and 42 antisense RNAs). The highest amount of differently expressed genes with outstanding statistical significance was observed in anaplastic carcinomas: 354 lncRNAs, including 140 lincRNAs and 109 antisense RNAs. In total, 26 lncRNAs were differently expressed in all histological subtypes.

Conclusion: Common and specific patterns of lncRNA expression are found in distinct subtypes of thyroid cancer.

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P17.63C
Genome-wide microRNA analysis of transient focal ischemia in rat brain

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Introduction: Ischemic stroke is one of the most serious diseases leading to death or disability of the population. The study of non-coding RNAs in ischemia has exceptional importance for the development of new strategies for neuroprotection and reconstruction of nerve tissues. It is known that microRNAs can play a role both as neuroprotective agents and also contribute to pathological processes in ischemia. The work is devoted to the study of the expression of miRNAs and their possible mRNA targets in rat model of cerebral ischemia with reperfusion (rMCAO), which resembles events in human ischemic stroke.

Materials and Methods: rMCAO in rats, high-throughput RNA sequencing (RNA-Seq), real-time RT-PCR, bioinformatics.

Results: A comparative analysis of the expression of microRNAs and mRNAs was performed in subcortical structures of the brain containing a damage focus in rMCAO. 24 h after occlusion, the expression level of 407 microRNAs was changed (Fold change>2, padj<0.05). Also, the expression level of mRNAs of 108 genes as potential targets for those microRNAs was changed. They included collagen (Col3a1, Col4a1), integrin (Itgb2, Itga5), dopamine receptor (Drd1, Drd2) mRNAs, and other genes associated mainly with signaling pathways Focal adhesion ($p = 1.4E-3$), ECM-receptor interaction ($p = 1.8E-3$), Gap junction ($p = 0.015$), and others.

Conclusions: RNA-Seq analysis revealed the active involvement of microRNAs in the regulation of inflammation, stress and neurotransmission in rMCAO. Further analysis of non-coding RNAs will facilitate the understanding of the mechanisms of post-transcriptional regulation of gene expression in brain ischemia.

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P17.64D

Altered microRNA profile in osteoporosis caused by impaired WNT signaling

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Introduction: WNT signaling is fundamental to bone health and its aberrant activation leads to skeletal pathologies. A heterozygous missense mutation p.C218G in *WNT1*, a key WNT pathway ligand, leads to severe early-onset and progressive osteoporosis with multiple peripheral and spinal fractures.

Subjects and Methods: A cross-sectional cohort study on 12 mutation-positive (35 years, range 9-59 years) and 12 mutation-negative (35 years, range 9-59 years) subjects from two Finnish families with a heterozygous p.C218G *WNT1* mutation. Serum samples were screened with a custom-designed panel of 192 miRNAs using qPCR. Findings were compared between the two groups.

Results: The pattern of 192 circulating miRNAs is significantly different in the mutation-positive subjects: two were upregulated (miR-18a-3p, miR-223-3p) and six downregulated (miR-22-3p, miR-31-5p, miR-34a-5p, miR-143-5p miR-423-5p, miR-423-3p) in the *WNT1* mutation-positive subjects (p-values=0.001-0.053). Three of these (miR-22-3p, miR-34a-5p, and miR-31-5p) are known inhibitors of WNT signaling: miR-22-3p and miR-34a-5p target *WNT1* mRNA and miR-31-5p is predicted to bind to *WNT1* 3'UTR.

Conclusions: The miRNA profile reflects *WNT1* mutation status. The results suggest that *WNT1* mutation disrupts a feed-back regulation between these miRNAs and WNT1, providing new insights into the pathogenesis of WNT-related bone disorders. These miRNAs could offer future potential in diagnosis and treatment of osteoporosis.

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P17.65A

Age-dependent patterns of X chromosome DNA methylation in the elderly

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The age-dependent patterns of DNA methylation in the elderly population have been intensively studied on the autosomal chromosomes, revealing large numbers of genomic sites under significant epigenetic remodeling during the aging process. However, DNA methylation changes on the X-chromosomes have been routinely ignored due to analytical difficulties in dealing with difference in X chromosome content of females and males and X chromosome inactivation in females. Taking advantage of large sample sizes of DNA methylation data available on the elderly, we performed X-chromosome-wide association study on X-linked DNA methylation changes during aging by analyzing male and female samples separately and comparing the rate of change in DNA methylation between sexes to identify significant CpGs sites differentially or consistently methylated in males and females. We detected 2300 significant CpGs (False Discovery Rate<0.05) displaying sex-independent methylation patterns and 286 CpGs showing sex-dependent methylation changes in the Lothian birth cohort born in 1936 in Scotland (732 males with mean age 71.27 and 732 females with mean age 71.23). As a replication approach, a similar analysis was performed in an independent sample of middle aged Danish twins (226 males with mean age 67.26 and 226 females with mean age 66.04) reporting 136 CpGs consistently and 59 CpGs differentially methylated in males and females with overlapping rates of 38.08% and 5.1% with the discovery results. In conclusion, we have shown significant DNA methylation patterns of aging on the X-chromosome dominated by sex-independent regulation highly replicable in independent samples.

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P18 Genetic epidemiology/Population genetics/Statistical methodology and evolutionary genetics

P18.02B
Investigating the population structure of Robinson Crusoe Island, Chile

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Robinson Crusoe (RCI) is a geographically and socially isolated island located 670km west of San Antonio, Chile. It was founded in 1876 by a group of eight founder families. It is now home to over 800 inhabitants, most of whom are descended from the original founder families. The geographical isolation of RCI means few outsiders have migrated to the island. As a result, islanders share a high degree of consanguinity (14.9%), and most islander unions are at least second cousins (Villanueva et al 2014).

RCI islanders show an exceptionally high occurrence rate of developmental language disorder, 10-fold the rate seen in mainland Chile. Near complete islander genealogical records have shown that 90% of affected children are direct descendants of a pair of original founder brothers, who likely carried a susceptibility variant for language disorders (Villanueva et al 2014).

To understand the genetic contribution to developmental language disorder on RCI, we have investigated the underlying population structure of the islanders. Extensive pedigree data suggest the current islander population are of recently admixed European and Chilean backgrounds. Recent studies have shown a higher proportion of indigenous South American ancestry in the mainland Chilean population than previously thought (Lorenzo-Bermejo et al 2017), and this may therefore be directly relevant to the RCI population.

Using high density genome-wide SNP genotyping data from 154 islanders, and whole genome sequencing from 24 of the most distantly related islanders, we have performed the first in-depth genetic analysis of the population structure of RCI.

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P18.04D
Analysis of bones ancient mtDNA from the medieval archeological site of Amiternum (L'Aquila), Italy

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Introduction: The study of ancient DNA allows to analyze genetic relationships between individuals and populations of the past and the present. In this work we analyzed human bones remains datable between the 6th-9th century D.C. from burials of the archaeological site of Amiternum, L'Aquila.

Materials and Methods: As a genetic marker, the hypervariable 1 region of mitochondrial DNA (HVR1)

has been chosen. The HVR1 marker has been amplified by PCR, the amplicon have been cloned and sequenced. Sequences of the HVR1 region were compared with Anderson's sequence for the identification of polymorphisms. The data obtained were analyzed with different software and phylogenetic methods. For inter-populations comparisons, the known sequences in literature and found in ancient and modern databases have been used.

Results and Conclusions: This work provides preliminary information on the correlation between the inhabitants of Amiternum and the Longobards populations of northern Italy and the Byzantines, migrant peoples transited and/or allocated in the territory of Amiternum. The study of the haplogroups, the analysis of genetic variability and the studies of phylogeny on the obtained sequences show a genetic proximity between individuals of Amiternum, the current population of north/central Italy and the Germanic tribe of Longobards, which dominated the Italian peninsula between 568 and 774 A. D. The match of ancient Byzantines sequences with one of the Amiternum samples highlights also a Byzantine genetic trait in the populations of Amiternum and L'Aquila. Grants RIA Univaq 2017 to Poma A and Redi F

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P18.05A

Prenatal diagnostics of anencephaly in the Czech Republic: 20 years of population wide surveillance

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Introduction: Anencephaly is a lethal congenital anomaly from the group of neural tube defects. Because of its distinctive phenotype, it is always diagnosed, either during pregnancy by ultrasonography, or clinically after the delivery. Our goal was to analyse the efficiency of prenatal diagnosis of this congenital anomaly in the Czech Republic.

Methods: We present retrospective epidemiological analysis of the prevalence of anencephaly in the Czech Republic. We used population based data from the National Registry of Congenital Anomalies, stored in the Institute of Health Information and Statistics of the Czech Republic (1996 - 2015 time period).

Results: During the selected 20 years a total of 592 cases of anencephaly were diagnosed (2.89 cases per 10 000 live births). Only 27 of those cases were diagnosed in births (0.13 per 10 000), the rest of cases were diagnosed prenatally (565 cases, 2.76 per 10 000). The average gestation week at the time of diagnosis was 17.21 in 1996 and 13.00 in 2015. The average maternal age changed from 23.68 years in 1996 to 28.64 in 2015.

Discussion: The majority of anencephaly cases are diagnosed prenatally in the Czech Republic. The overall percentage of terminations of pregnancy is high (95.44%). We also observed an improvement in ultrasound based prenatal diagnosis along the study period, leading to a significant decrease in the average gestation week at the time of diagnosis.

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P18.06B

Rare coding variants and the risk of congenital anorectal malformations: an exome chip association study

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Introduction: Anorectal malformation (ARM) is a rare birth defect, resulting from disturbed development of the hindgut. Although suspected, evidence of a genetic etiology is still scarce. The aim of this study is to identify rare variants in ARM etiology.

Materials and Methods: We genotyped 568 Caucasian ARM patients and 1,860 population-based controls using the Illumina ExomeChip, which contains >240,000 rare coding variants. GenomeStudio clustering and calling was followed by re-calling of 'no-calls' using Zcall, for patients and controls simultaneously. Single variant analyses were performed to identify statistically significant variants (Bonferroni corrected threshold $p<1.15*10^{-6}$). Based on the calling quality in the clusterplots and distribution of minor alleles for the variants among patients and controls, variants were identified for validation using Sanger Sequencing.

Results: In total, 13 single variants reached statistical significance. However, the majority of minor alleles for these variants were absent in controls, and some patients showed 3 or more minor alleles for these 13 variants, which is highly unlikely. We identified the 3 most promising candidate variants with acceptable calling quality that remained statistically significant in the single variant analysis after exclusion of patients with 3 or more minor alleles. However, Sanger sequencing did not confirm presence of the minor alleles for the variants in patients as indicated based on ExomeChip data.

Conclusions: We did not find evidence for associations between ARM and rare coding variants present on the ExomeChip. Furthermore, we would like to emphasize the importance of validation of ExomeChip data before large replication studies are initiated.

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P18.08D

Genetic and molecular analysis of urinary magnesium concentration in Scottish and Croatian populations

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Introduction: Electrolyte imbalance, including changes in magnesium concentration and other electrolyte ratios in the body can result in dizziness, arrhythmia and if left untreated can result in serious illness or even death. Magnesium is the second most abundant bivalent cation in the body and is essential for many cellular processes. Renal magnesium handling plays an important role in maintaining magnesium homeostasis, however the exact biological mechanisms remain unclear. A recent genome-wide association study (GWAS) identified an association between urinary magnesium concentration (uMg) and variants in the *ARL15* gene on chromosome 5. *ARL15* encodes a GTP-binding protein that interacts with the magnesium transporter channel TRPM6, and other proteins involved in magnesium homeostasis in physiologically relevant cell lines.

Materials and Methods: We conducted meta-analyses for urinary magnesium, magnesium to creatinine (uMg/cre) ratio, as well as magnesium concentration to other clinically relevant electrolyte ratios in 11, 617 individuals from Scottish and Croatian populations to determine possible genes involved in these traits.

Results: We observed genome-wide significant peaks in the *ARL15* locus in our meta-analyses conducted for uMg, magnesium to creatinine (uMg/cre), magnesium to phosphate (uMg/uPh) and magnesium to potassium (uMg/uK) ratios. The top SNP associated with uMg and uMg/cre in the meta-analyses lies within a transcription factor binding site in an enhancer region of *ARL15*.

Conclusions: We hypothesise that the variant found affects the expression of *ARL15*, which, in turn modulates magnesium transport. We are performing functional studies to elucidate the role ARL15 plays in magnesium homeostasis both *in vitro* and *in vivo*.

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P18.10B

Identification of incompletely penetrant variants by a population genetic approach

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Autosomal recessive (AR) disorders are typically completely penetrant. We recently identified the first variant (*NPHS2*, p.R229Q), that is pathogenic only with specific trans-associated mutations. We aimed to identify other incompletely penetrant variants (IPV) in AR disorders. We collected clinical and genotype data from 9.283 Caucasian patients with biallelic HGMD mutations in 19 genes responsible for frequent AR disorders (*ASL, ATP7B*, *CAPN3*, *CFTR*, *CTNS*, *DHCR7*, *GAA*, *GALNS*, *GALT*, *IDUA*, *MUT*, *NPHS1*, *NPHS2*, *PAH*, *PCCB*, *PKHD1*, *PMM2*, *SLC26A4*, *TYR*) in the medical literature. Variants that were less frequent in the patient population than in the general European non-Finnish population of gnomAD were considered non-pathogenic and were excluded (n=28). The penetrance of each pathogenic variant (n=2141) was calculated as its enrichment in the patient population as compared to that of the loss-of-function (LOF) mutations. *DHCR7* and *PMM2* LOF mutations were underrepresented in the patient population suggesting in utero lethality. Out of the 82 variants that were frequent enough to assess their penetrance, we found 23 (28%) *ASL*, *CAPN3, CFTR, GAA, NPHS2, PAH* and *PKHD1* variants to be incompletely penetrant, including the known IPVs (*NPHS2* R229Q, *CFTR* R117H and L997F). No other but the R229Q variant was subject of interallelic interactions. Based on the associated phenotype, most of the IPVs are hypomorphic (16/23, 70%) vs. 19/59, 32% of completely penetrant variants, $p = 0.0002$). Frequent IPVs can be identified by this computerized population-genetic approach and are more common than expected. Proper genetic counseling requires their knowledge. This work was supported by MTA-SE Lendulet Research Grant (LP2015-11/2015).

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P18.11C

Barrett's esophagus and esophageal adenocarcinoma: systematic integration of eQTL data and candidate loci association study

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Barrett's esophagus (BE) is a metaplasia at the lower esophagus and a precancerous condition for the development of esophageal adenocarcinoma (EA). The etiology of both BE and EA is multifactorial. A recent GWAS meta-analysis has led to the identification of 14 susceptibility loci for BE/EA. The aim of the present study was (i) to link the genome-wide genetic data of the meta-analysis with gene expression levels through expression quantitative trait loci (eQTLs) and (ii) to analyse identified eQTL-SNPs in an independent cohort.

For this, the meta-analysis data were systematically integrated with the eQTL data from the GTEx tissues from gastroesophageal junction and esophageal mucosa. We selected SNPs that (i) revealed a p-value of $5\times10^{-5}>p>5\times10^{-8}$ in the BE/EA meta-analysis and (ii) were an eQTL in at least one of the esophageal GTEx tissues. Here, we identified 35 eQTLs. Of these, 32 were integrated into an independent genotyping study, consisting of 1,406 BE/EA cases and 992 controls. Following quality control, in which 9 SNPs were excluded, four SNPs showed a nominal significant association. In a next step, we performed a fixed-effect meta-analysis. The best association P-value was observed for the SNP rs37050130 ($p = 9.22\times10^{-8}$), an eQTL for AF131215.9, which is an uncharacterized gene. The second best associated eQTL mapped to *SLC22A3*, encoding an organic cation transporter that improves chemotherapy drug absorption.

Our study is the first systematic approach that identifies specific eQTLs at BE/EA candidate loci and it provides first insights into the biological mechanism contributing to the development of BE/EA.

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P18.12D
Multi-phenotype genome-wide meta-analysis of lipid levels and BMI in 64,736 Europeans suggests shared genetic architecture

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Serum lipid levels and obesity share biochemical pathways, suggesting overlapping causal genetic factors. Genome-wide association studies (GWAS) of correlated phenotypes have been developed to identify such shared genetic effects with increased power.

We performed a multi-phenotype GWAS (MP-GWAS) on three blood lipids (high-/low-density lipoprotein cholesterol and triglycerides, HDL-C/LDL-C/TG) and body-mass index (BMI) in 22 European-ancestry studies (N=64,736) imputed to the 1000 Genomes reference panel (Phase 1). We fitted a "reverse regression" model between each single-nucleotide variant (SNV) and the linear combination of HDL-C/LDL-C/TG/BMI using the SCOPA software, i.e. for the ith variant $SNV_i=\beta_{1i}\times HDL\text{-}C+\beta_{2i}\times LDL\text{-}C+\beta_{3i}\times TG+\beta_{4i}\times BMI+\varepsilon_i$, where $\varepsilon_i \sim N(0,\sigma^2)$. Study-specific effect sizes and variance-covariance matrices for each variant were combined in a meta-analysis using the META-SCOPA software.

We identified 14 novel and 41/9 established lipid/BMI loci, respectively, at genome-wide significance ($P < 5x10^{-8}$). Nine novel (*SDC1*, *SLC8A1*, *EPHA6*, *SPATA4*, *MAGI2*, *CTSB, BC014119, SMCO4* and *CNTN5*) and 32 established loci showed effects on both BMI and lipids in the joint model, suggesting shared genetic architecture. This observation was supported through hierarchical cluster analysis, which resulted in six clades representing a mixture of lipid- and BMI-associated variants. We detected significant eQTL effects in whole blood, subcutaneous/visceral fat and liver at 16 of the identified loci, and enrichment of association signals at HDAC6 binding sites, indicating a critical role of associated loci in various cellular events.

The MP-GWAS is a powerful approach to detect shared genetic effects on correlated phenotypes, as demonstrated by our analysis of lipids and BMI.

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P18.13A
Potential adaptive role of the human-specific duplication at 16p11.2 in iron homeostasis and immune response

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Recurrent pathogenic copy-number variation (CNV) at human chromosome 16p11.2 is mediated by *Homo sapiens*-specific duplications of a segment including the *BOLA2/2B* and *SLX1A/1B* genes. This segment is copy-number variant in humans (3-8 copies per genome) and its duplication is almost fixed most likely due to positive selection. BOLA2-GLRX3 heterotrimers participate in iron homeostasis. SLX1, together with SLX4, MUS81 and EME1, forms a complex involved in the control of genome stability. *SLX4* mutations cause Fanconi anemia, characterized by the inability to produce blood cells.

To gain insights into the potential adaptive role of this human-specific duplication, we analyzed hematological and blood iron data in 16p11.2 deletion carriers with varying copies of this segment. We found that 50% of individuals with the lowest number of copies, i.e. three, need iron supplementation and/or are anemic, and 4 out of 6 have low lymphocyte counts. When compared to individuals with more copies, i.e. four and five (n=14), the frequency of the iron phenotype was not significantly different (P=0.13) while the low lymphocyte count was (P=0.004). Similarly, mouse models carrying the 16p11.2 orthologous deletion

and, more specifically, *Bola2* knockout mice show significantly lower blood iron and hemoglobin levels than their wild-type littermates. Corroboratingly, gene dosage of the 16p11.2 BP4-BP5 CNV is positively associated with blood lymphocyte count in the UK biobank cohort (P=0.007).

Taken together, our results suggest a role of the genes mapping to this human-specific duplication in iron homeostasis and lymphocyte level and a potential adaptation in improving iron metabolism and/or immune response.

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P18.14B

What is the significance of 15q11.2 BP1-BP2 deletions and duplications?

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Purpose: To assess the detection rate of copy number variations (CNVs) of 15q11.2 (BP1-BP2) in relation to indications for testing, and other clinical characteristics.

Methods: We analyzed 11,004 chromosomal microarray tests performed in a single referral lab during a four-year period (7,596 prenatal, 3,408 postnatal).

Results: Deletions were detected in 66 cases (0.6%); 39 in prenatal tests (0.51%) and 27 in postnatal tests (0.79%). Duplications were detected in 94 cases (0.85%); 62 in prenatal tests (0.82%), 32 in postnatal tests (0.94%). The prevalence of deletions and duplications among clinically-indicated prenatal tests was 0.57% and 0.9% respectively, which was not statistically different from tests performed without an indication; 0.49% and 0.78% ($p = 0.6$, 0.59, respectively). The prevalence of deletions and duplications among postnatal tests performed due to a clinical indication was 0.73% and 1%, respectively, which was similar to the prevalence in healthy individuals; 0.98% and 0.74%, respectively; ($p = 0.497$, 0.67). There was no difference between fetal sex, the prevalence of additional CNVs and the size of the deletion/duplication involving 15q11.2 between indicated and unindicated tests.

Conclusions: 15q11.2 (BP1-BP2) deletions and duplications are common findings among affected and unaffected populations, suggesting a low pathogenicity of these CNVs.

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P18.15C

Study of the character of psychiatric symptoms with hereditary metabolic diseases. Symptom complex and comorbidity

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Introduction: F.Sedel's classification of CMD according to the type of psychiatric symptoms in disease onset allows to adequately estimate the nature of polyorganous affections, clarify the diagnosis and, normalizing metabolism, relieve the patient from psychiatric disorders.

The Objective: to study CMD spectrum, accompanied by psychiatric disorders.

Results: among first applied 132,309 patients, 1056 nosological forms were revealed. Children with mental retardation - 863 cases - 0.65% among the first-applied, and 81% among the nosological forms. Psychiatric diseases - in the case of urea formation cycle defects, methionine remineralization defects, porphyria. Chronic psychiatric symptoms - in patients with homocystinuria, Wilson's disease, adrenal leukodystrophy and lysosomal accumulation diseases. Homocystinuria, spinal-tendinous xanthomatosis, non-ketone hyperglycinemia, monoamine oxidase insufficiency, insufficient dehydrogenase of half-aldehyde of succinic acid and creatine transporter deficiency - in patients with mild mental retardation and behavioral changes. The greatest number of patients with these changes - in porphyria and methionine remethylation disorder. In severe cases of tryptophan, methionine, heme metabolism, comprehensive treatment has allowed to get mental recovery.

Conclusions: since 2007 - the conduction of selective screening for methionine metabolism, investigating the polymorphisms of MTHFR, MTRR, MTR and RFC1 folate carrier. 11 579 patients were examined in whom heredity is burdened by cardiovascular, psychiatric, oncological disorders. Population frequency of homozygotes C677T MTHFR - 7.04%, among patients - 10.1%; the allele frequency of MTRR A66G - 57%. These features increase the chances of combining any form of CMD with folate-methionine cycle disorder. Currently we determine comorbidity frequency.

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P18.16D

CNVAssociation with Neurodevelopmental Phenotypes in Finnish Population Cohort

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Copy Number Variations (CNVs) are associated with a myriad of syndromic and severe developmental disorders, often overlapping developmentally important genes. Usually considered high-impact variants, CNVs are nevertheless also observed in the general population, where their impact is poorly characterised and knowledge of their impact to health and well-being is limited. To investigate the effect of CNVs on the health, risk for neurodevelopmental phenotypes and socioeconomic and educational attainment in the population in general, we employ longitudinal health record data together with genotypic information from 20 098 individuals in the FINRISK population cohort. Phenotype information includes: neurodevelopmental diagnoses from health record data spanning over 35 years; use of select classes of prescription drugs (data spanning 20 years); and socioeconomic factors reported on participation forms. Genotypic information entails intensity and allelic frequency data retrieved using 542 585 probes in the Illumina HumanCoreExome DNA MicroArray. CNVs are identified using both PennCNV and iPsychCNV, focusing initially on CNVs identified by both algorithms. All calls will be manually curated for validity. We have analysed the dataset with PennCNV, the iPsychCNV analysis and phenotype association analyses are ongoing. In our preliminary analysis, carrying a large deletion (>1Mb) increased the risk for a neurodevelopmental or neuropsychiatric phenotype almost 2-fold (18.6% vs 9.8%). For intellectual disability, the risk ratio was 9.6 (2.3% vs 0.24%). We will also perform enrichment analyses for neurodevelopmental phenotypes, use of prescription drugs, socioeconomic status, educational attainment and overall health status.

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P18.17A

Targeted resequencing of non-coding risk loci using single-molecule molecular inversion probes (smMIPs)

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The genetic etiology of complex traits is largely characterized by associations of common risk variants located in non-coding regions. These regions might also harbor putative causative, rare variants in individual families, which can be identified by targeted resequencing using smMIPs. These are 75 bp-long molecules composed of two region-specific primers that are linked by a common backbone sequence. Upon contact with genomic DNA, smMIPs form a circular DNA fragment around the target region. Barcoded primers enable multiplexing, and unique molecular tags in the smMIPs backbone reduce the number of PCR artifacts in downstream analyses. In a first assay, 19 non-coding candidate regions at four risk loci for non-syndromic cleft lip with/without cleft palate were selected for resequencing in 1,061 cases and 1,591 controls. Data analysis included smMIP-design by MIPGEN, read alignment with BWA and variant calling with UnifiedGenotyper. Downstream annotation was based on rarity of variants, annotation scores (e.g. CADD, FATHMM-MKL, LINSIGHT, DANN, and Eigen), and co-segregation analyses in family members. We also assessed concordance rates between genotypes obtained by array genotyping and smMIPs, and identified a concordance of 100% for each of three analyzed common SNPs in 1,400 samples, after filtering out low-quality smMIPs variant calls. First annotation results show varying degrees of correlation between the different scores, suggesting that no single score is sufficient for assessing the potential pathogenicity of variants. We suggest that smMIPs resequencing is a powerful tool for targeted sequence analysis beyond the protein-coding regions, in large cohorts, in a cost-efficient way.

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P18.20D

Genetic homogeneity in DARWIN, an isolated population in the Netherlands

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Introduction: Isolated populations have the potential to facilitate gene mapping of complex traits. The number of haplotypes shared among inhabitants is smaller, while the length of the shared haplotypes is longer, as they have been derived from a handful of relatively recent founders. Together with genetic drift and restricted migration this leads to the enrichment of rare alleles and genetic homogeneity. Combined with environmental homogeneity resulting from a comparable lifestyle, population isolation increases power to detect genetic risk loci.

Methods and Results: The DARWIN (*DNA Analysis in Residents Within an Isolate in the Netherlands*) village is an isolated population (~22.000 inhabitants) that was founded in the 14th century by 7-20 families, and remained in religious and cultural isolation ever since. By using whole genome sequencing data of 20 DARWIN trio's, we show that the DARWIN population has a high inbreeding coefficient, expressed as F_{ROH}: the percentage of the autosomal genome that is in runs of homozygosity (ROH). DARWIN shows features of ancient inbreeding (F_{ROH} 3.2% with an ROH threshold of 0.5 Mb) and recent inbreeding (F_{ROH} 1.3% with a threshold of 5 Mb). These numbers are ~6-14 times greater than the F_{ROH} of the overall Dutch population, represented by 748 trio's from the *Genome of the Netherlands* study. Furthermore, the F_{ROH} is equal to or even higher than that of previously described population isolates, such as Sardinia.

Conclusion: The DARWIN village is a population isolate whose genetic homogeneity offers great potential for future genetic studies.

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P18.21A

Age, origin and spreading of the myotonic dystrophy type 2 mutation throughout Europe

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Introduction: Myotonic dystrophy type 2 (DM2) is mainly a disease of the European Caucasians. Haplotype analyses suggested a founder mutation ~4000-11000 years old, which coincides with the Neolithic Age, when the Near Eastern farmers, and afterwards herders from the Pontic-Caspian steppe, had settled in Europe, shaping the genomic architecture of the Europeans. We aimed to estimate the age

and origin of DM2 mutation and reconstruct its dispersal route.

Materials and Methods: *CL3N122*, *CL3N99*, *CL3N59*, *CL3N119*, *CL3N19* and *CL3N23* loci were genotyped in 401 individuals from Serbian, Greek, Slovenian, Slovakian and Czech DM2 families. 320 healthy and 79 DM2 haplotypes phased by family segregation analysis, and 53 DM2 published German haplotypes were used for the coalescent modeling of intra-allelic variability in DMLE+. The maximum likelihood estimation (MLE) of the mutation age was determined for the population growth rate set at 0.025-0.05, assuming DM2 mutation frequency in Finland (1/1830).

Results: The estimated DM2 mutation age is 170-280 generations (~3400-5600 years assuming 20 years/generation). It dates back to the Late Neolith and the early Bronze Age when massive migrations of Yamnaya individuals from the Pontic-Caspian steppe to Europe occurred (3500-2200 BCE), accompanied by an expansion of the Corded Ware individuals (2800-2200 BCE).

Conclusion: Presented results bring a novel insight into the origin and spreading of DM2 mutation throughout Europe. According to epidemiological data, distribution of the DM2 mutation seems to reflect a decreasing Yamnaya ancestry from the north to the south in the present-day Europeans.

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P18.22B

Changing frequencies of main autosomal trisomies in the Czech Republic: population-based data

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Introduction: Down, Edwards and Patau syndrome are the most common chromosomal abnormalities diagnosed during the prenatal diagnostics. The major goal of this study was to identify any possible trends that can be associated with the gradual implementation of non-invasive prenatal genetic testing in the Czech Republic.

Methods: The population-based data on prenatally diagnosed cases were obtained from the National Registry of Congenital Anomalies of the Czech Republic (2012-2016 time period). The proportion of the number of the three major autosomal trisomies was compared to the proportion of all other chromosomal aberrations that were identified during prenatal diagnostics. The annual number of live births in the Czech Republic was used as a denominator. The ratios were subjected to the logistic regression. Statistical evaluation was carried out by the statistical software R.

Results: Down, Edwards and Patau syndromes together presented 62.6% of all prenatally diagnosed chromosomal aberrations. The overall frequency (per 10 000 live births) of major autosomal trisomies have increased from 30.86 in 2012 to 37.41 in 2016 (Poisson regression: $p = 0.014$). Also the proportion of major autosomal trisomies increased from 57.22 % (of all prenatally diagnosed aberrations) in 2012 to 66.09 % in 2016 (logistic regression: $p < 0.001$).

Discussion: We confirmed that the overall frequency of major autosomal trisomies (and their proportion) is increasing. The possible explanations are the improving methods of prenatal diagnostics and also the increasing maternal age in the Czech Republic.

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P18.23C

Epidemiology of Down syndrome in Argentina

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Introduction: Down syndrome (DS) is the most common chromosomal disorder in children. Purpose: To describe the epidemiologic characteristics of DS in Argentina based on data from the National Network of Congenital Anomalies (RENAC).

Materials and Methods: The prevalence of DS by province and by maternal age for the period 2009-2015 was calculated, and compared between healthcare system sectors (private vs. public) and complexity levels of public hospitals. The proportion of cases with prenatal diagnosis was established. The association between DS and lower birth weight and gestational age was analyzed.

Results: From 1,358,158 births examined, 2,344 cases of DS were registered. The prevalence -expressed per 10,000 births- was 17.26; among provinces it varied between 10.99 and 23.71. By maternal age, the prevalence ranged from 10.32 in <20 years, to 158.06 in those ≥45. In public hospitals the prevalence was 16.25 and 21.55 in private hospitals, prenatal detection was 14.1% and 30.5% respectively. In high complexity public hospitals the prevalence was 17.45 and 14.62 in hospitals with lower complexity. There was a negative correlation between birth weight and DS (B:-362.22, p<0.01). The mean gestational age was 0.28 weeks lower in DS newborns than in the general population.

Conclusions: DS prevalence in Argentina is similar to that reported previously in the region. A higher prevalence and percentage of prenatal diagnosis was observed in private hospitals. The association between DS and lower birth weight is consistent with previous findings. Knowledge of the epidemiological characteristics will allow the implementation of health policies for this pathology.

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P18.24D

Relationship between effective population size and inbreeding in the Lithuanian population

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Introduction: In previous studies, we have reported low effective population size (*Ne*) and census population size (*N*) ratio in the Lithuanian population. The estimates of *Ne* were approximately one-tenth of the Lithuanian census size compared with other genetics-based estimates of between 0.21–0.65. Natural levels of fluctuations such as variance in size, reproduction, sex ratio, the degree to which generations overlap, and possibly inbreeding caused the small values of *Ne/N*. The main interest of this study was to evaluate the potential impact of small *Ne/N* on inbreeding, estimating the inbreeding coefficient (F_t) for 50 generations from effective population size data of Lithuania.

Methods: 295 randomly selected DNA samples were genotyped using the Illumina 770K HumanOmniExpress-12v1.0 array. The *Ne* values were obtained for 50 generations assuming a generation time of 25 years by using inferred long segments of identity by descent (IBD) from high-density genotyping data. The inbreeding coefficient in generation t was estimated as

Results: The estimated Inbreeding coefficient range from 0.00209 in generation 50 to $1.305 \cdot 10^{-6}$ in generation one, with average value of 0.000645. As was expected we observed F_t decrease as *Ne* increased. Statistically significant difference between F_t values of generations and average value was not found ($p = 0.5964$, Single sample Wilcoxon Test).

Conclusion: The results don't confirm the effect of elevated inbreeding as couse of small *Ne*/N in the Lithuanian population. However, more detailed analysis is needed.

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P18.25A

The eQTLs Catalog and LinDA browser: a platform for prioritising target genes of GWAS variants

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The expression Quantitative Traits Loci (eQTLs) are genetic polymorphisms associated with changes in gene expression levels. They have been successfully used to prioritize the target genes of the variants associated with complex traits and diseases (GWAS variants). Up to date a few eQTLs

databases exist and they collect only a small portion of the available datasets.

We thus planned to build the largest publically available catalog of eQTLs, coupled with a browser, to optimize and simplify their interrogation.

We collected and manually curated 51 eQTL public studies ranging from 2007 to date, corresponding to more than 100 sample types and 25 human populations for a total of 259176 cis-eQTLs and 32929 genes with at least one cis-eQTL (cis-eGenes). Most of the eQTLs studies were conducted in blood samples from healthy individuals of European ancestry. We found that for 93% of the known protein-coding genes were eGenes, 20% of them intersecting ($r^2 \geq 0.8$) with the NHGRI-EBI GWAS Catalog and 26% of whom considered as druggable. Furthermore, for those GWAS variants for which an eGene was known, we found that the NHGRI-EBI GWAS Catalog proposed the same gene as candidate target only for the 60% of the times.

Our eQTL-Catalog can be used as a reference to measure the degree of novelty for future eQTLs studies; it is provided within a platform with a web interface (LinDA) that we plan to implement with other types of quantitative traits (i.e. epigenetic, proteomic, metabolomics and microbiota) to better dissect the pleiotropy of the GWAS variants.

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P18.26B

Bioinformatic and mendelian randomization analyses characterize variants in desmosomal genes associated with ECG traits in the general population

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Introduction: Arrhythmogenic cardiomyopathy is caused by mutations in desmosomal genes. We previously tested association of common variants in such genes with physiological cardiac conduction traits. From an analysis of 4342 subjects from the Cooperative Health Research In South Tyrol study, we reported association of two Junction Plakoglobin (*JUP*) SNPs with P wave length and one Desmoplakin (*DSP*) SNP with QRS interval.

Material and Methods: In this subsequent study, we assessed replication in the MICROS (N=636), an independent study from the same geographical region, and in the Northern Germany SHIP/SHIP Trend (N=3797) studies. We characterized the variants and their genomic context using the SCREEN/ENCODE tool, the GTeX and methylation QTL (meQTL) databases, and Framingham Heart Study meQTL data (N=4170). We tested few biological hypotheses using two-sample Mendelian randomization (MR) based on Wald estimator, with standard errors computed via delta method.

Results: The *DSP* rs2744389-QRS association was replicated in MICROS ($p = 0.010$) but not in SHIP/SHIP Trend ($p = 0.505$), suggesting genetic heterogeneity. We did not replicate the P wave associations. Falling in the *DSP* promoter, the rs2744389 is an eQTL of the antisense RNA RP3-512B11.3, and a meQTL of cg02643433. MR analysis showed evidence of a causal effect of cg02643433 methylation on QRS ($p = 0.001$), of RP3-512B11.3 expression on QRS ($p = 0.030$), but lacking evidence of a causal effect of RP3-512B11.3 expression on cg02643433 methylation ($p = 0.090$).

Conclusions: While needing to understand the reasons for the partial lack of replication, MR results suggest an antisense RNA-mediated mechanism of DSP expression control to be validated with laboratory experiments.

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P18.27C

A functional strategy to characterize expression Quantitative Trait Loci

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Introduction: Expression Quantitative Trait Loci (eQTL) studies represent an important step in the functional characterization of genetic variants that have been associated with diseases by Genome Wide Association Studies (GWAS). We propose a new strategy to detect eQTLs leveraging the idea that changes in gene expression often pass through the alteration of transcription factors (TFs) binding on regulatory regions.

Material and Methods: The Total Binding Affinity (TBA) measures the propensity of a TF to bind a sequence. In each model, we compute TBA values for 640 human TFs on a regulatory region after the reconstruction of its sequence in several individuals and then correlate this TBA profile with the expression of a target gene through principal component regression. This approach was applied to the analysis of a dataset including genome and coupled gene expression data for 344 European individuals.

Results: Our TBA-based inference detected 3,781 eQTLs, of which 1,543 weren't revealed by the traditional single-SNP method. In addition it permitted the formulation of mechanistic hypotheses pinpointing for each gene the most relevant TFs in each associated regulatory region and we showed that these outcomes are fairly consistent with the presence of binding QTLs (bQTLs).

Future perspectives: We want to integrate the TBA model with new approaches, collectively named Transcriptome Wide Association Studies (TWAS), that combine GWAS and eQTL data to identify susceptibility genes. At least in principle, TBA-based TWAS have the advantage of considering also the effect of rare variants that cannot be measured in standard eQTL studies.

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Complement *C1Q* genes act as modifiers of age-at-onset in TTR-FAPVal30Met Portuguese patients

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Introduction: Transthyretin (TTR) familial amyloid polyneuropathy (FAP) (OMIM 176300) shows a variable age-at-onset (AO), including within some families. We hypothesized that candidate genes associated with TTR pathways, such as *C1QA* and *C1QC*, might act as genetic modifiers of AO in TTR-FAP Val30Met Portuguese patients.

Subjects & Methods: We analysed DNA samples of 267 patients (117 families). To search for variants, all exons and flanking regions were genotyped by automated bidirectional sequencing. We used generalized estimating equations (GEEs) to take into account the non-independency of AO among relatives. Intensive *in silico* analyses were performed, using various software to assess miRNAs target sites, splicing sites, transcription factor binding sites alterations and gene-gene interactions.

Results: Two variants for *C1QA* gene: GA genotype of rs201693493 ($P<0.001$) and CT genotype of rs149050968 ($P<0.001$) were significantly associated with later AO (≥ 50 years). *In silico* analysis demonstrated, that rs201693493 may alter splicing activity. Regarding *C1QC*, we found three statistically significant variants: GA genotype of rs2935537 ($P=0.003$), GA genotype of rs201241346 ($P<0.001$) and GA genotype ($P<0.001$) of rs200952686 ($P<0.001$). The first two were associated with earlier AO (≤ 40 years), while the third was associated with late-onset.

Conclusions: *C1QA* was associated with later. *C1QC* may have a double role: variants may confer earlier or later AO, depending on the associated pathophysiological mechanisms. We thus confirmed the role of *C1Q* complement genes as modulator of AO in TTR-FAP Val30Met Portuguese patients.

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P18.30B

Association of genetic markers of coagulation and fibrinolysis with prematurity complication

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Introduction: The risk of complications in premature babies increases with lower gestational age (GA) and lower birth weight (BW). Most frequent complications are respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH),

retinopathy of prematurity (ROP) and sepsis. Recent studies showed that FV Leiden and PAI-1 4G/5G gene polymorphisms are associated with spontaneous abortion and prematurity. Our objective was to analyse the FV Leiden and PAI-1 4G/5G variants in order to determine their association with complications in premature babies.

Material and Methods: The study included 136 premature babies, mean GA= 30,46±1,98 and BW=1294 ±278,3. Genotyping of FV Leiden variant was performed by Real-time PCR analysis with specific TaqMan assay. PAI-1 4G/5G variants genotyping was performed by PCR-RFLPs method.

Results: The frequency of complications in premature babies was: 73,7% RDS, 19,1% BDP, 14,6% IVH, 46,7% ROP and 35,8% sepsis. The frequency of risk allele A for FV Leiden variant is 6.7%. FV Leiden variant is associated with development of BPD (X^2=5,245, p = 0,022; OR 0,302, 95% CI 0,104-0,877). The frequency of risk allele 4G for PAI-1 4G/5G variant is 60,6%. PAI-1 variant is a associated with IVH expression (X^2=6,700, p = 0,035; 0,257, 95% CI 0,088-0,775). There was no significant association of analyzed variants with RDS, ROP or sepsis.

Conclusion: Our findings suggest that FV Leiden variantis a risk factor for development of BPD while the presence of 4G variant is associated with occurrence of IVH.

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P18.31C
Use of large pedigrees from the FarGen project to gain information on consanguinity and inbreeding within the Faroese population

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Introduction: The Faroe Islands is a north Atlantic isolate with a current population size of 50.000 individuals. The Multi-Generation register of the Genetic Biobank (www.biobank.gov.fo) comprises hereditary records of all individuals registered in the Faroe Islands since 1800, moreover the majority of the individuals (85%) have records dating back to 1650. Together with the FarGen project (www.fargen.fo) we are using this valuable resource of genealogy to show consanguinity and inbreeding within the Faroese population.

Materials and Methods: In accordance with the FarGen project pedigrees were made from all 1530 individuals who had registered as FarGen participants. All the participants are in the Multi-Generation register and have answered a questionnaire about health status. Three different pedigrees were constructed: 1) all 1530 participants, 2) participants with "good" health status, and 3) participants with "bad" health status. From the 21 different ICD-10 groups of diseases reported in the FarGen project, pedigrees were made using 15 specific diseases.

Results: We present three large pedigrees spanning seven generations, with data from group pedigrees with good and bad self-reported health status we show whether consanguinity reflects the outcome, and trace its origins to the different islands on the Faroe Islands. We present 15 family pedigrees with specific disease status, which may contribute to our understanding of the pathogenesis of these diseases.

Perspectives: The pedigrees will be used in combination with genotype data to gain information on population stratification and the demographical history of the Faroe Islands.

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P18.32D
Households and Family Projections: Tehran Lipid and Glucose Study: 1999 to 2016

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Introduction: During last decade, along with developing high throughput sequencing technology and mapping the human genome, social and biological relationships are considered as pivotal tools in medical genetics. Family-based studies are suitable for population stratification control, investigations of parent-of-origin; X-linked transmitted inheritance and identification of disease risk factors. The aim of the current study was to describe the family structures of Tehran Lipid and Glucose Study (TLGS) population, and rates of its consanguineous marriage.

Materials and methods: The TLGS is one of the oldest longitudinal cohort study, which has been performed on a

population residents of the region district 13 of Tehran municipality, in two main and four follow up phases. Between 1999-2016, the complete genealogy data of 20,077 participants were documented and collected. Pedigree of each participant was drawn on paper sheet based on standardized human pedigree nomenclature and checked by ChIP-Typed data from the Tehran Cardiometabolic Genetic Study (TCGS).

Results: Genealogy data of participants and their relatives overall for 23259 individuals were checked through 5434 households. Before pedigree drawing and splicing unrelated individuals, the mean of cluster was 4.2 ± 1.9 (Min 1, Max 18). After taking into account ChIP genotyping data, 1741 clusters which their members had a common ancestor, joined together to make extended families by the appropriated methods. With splicing and adding disjointed, dummy individuals, 25642 remained in 3724 ± 6.9 (Min 3, Max 52) pedigrees. Family structures of the TLGS along with demographic, anthropometrics, biochemical, genetics, and well-phenotyped data can explore more genetics different.

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P18.33A

A genome-wide gene by lifestyle interaction study on high blood pressure

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Introduction: Epidemiologic studies showed synergistic effects on the risk of atherosclerotic cardiovascular disease due to the interplay between high blood pressure (HBP) and lifestyle including smoking, alcohol, and obesity. Although several GWASs and candidate-gene studies have found out GxE interactions on hypertension, relatively fewer studies have been performed to scan the entire genome to identify variants that modify the effects of lifestyle on the risk of HBP.

Methods: 21,504 individuals from three Korean cohorts were involved. Blood pressure was measured following the American Heart Association (AHA) guideline. We conducted exhaustive GxE interaction analyses for 4.1 million SNPs imputed using the 1000 genomes GRCh37 reference panel. We applied various exhaustive scans and two-step methods for detecting GxE interactions in parallel: step-wide penalties according to marginal *p*-value were applied for two-step methods.

Results: We identified five loci near *ABCG8*, *ATP2B1*, *CWC27* including the genetic interaction with smoking. The heritability explained by *ABCG8* was 0.9% by a GWAS but was increased to 5.7% when we considered GxSmoking interactions. We also identified 11 significant loci near *CCDC63*, *SH2B3*, *CUX2* for GxAlcohol interactions. About 11.6% of the heritability has been elucidated further due to the interplay between identified loci and alcohol. We identified several suggestive loci that synergistically increase the risk of HBP with obesity.

Conclusion: The identified loci might be used as genetic markers to give a personalized guideline for those who are particularly susceptible to smoking, alcohol, and obesity. Some amount of missing heritability would be explained by discovering more GxE interactive loci.

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P18.34B

Genetic landscape of kidney function: results from a trans-ethnic genome-wide association meta-analysis of >750,000 individuals

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Introduction: Chronic kidney disease (CKD) is a public health threat, which affects >10% adult population in Western countries and is associated with increased risk of end-stage renal disease, cardiovascular disease, and all-cause mortality. In the absence of therapies for CKD prevention, understanding the biological regulators of glomerular filtration rate (GFR), the key element that defines CKD, is of the highest relevance.

Materials and Methods: Within the CKDGen Consortium, 121 studies applied standardized protocols and scripts to run genome-wide association scans (GWAS) of estimated GFR (eGFR) on ~9 million high-quality SNPs, imputed on the 1000 Genomes ph3v5 or Haplotype Reference Consortium datasets. We performed fixed-effect meta-analysis of GWAS data on >750,000 participants of European, East-Asian, South-Asian, Hispanic, and African ancestries. Effect heterogeneity due to ancestry was investigated using trans-ethnic genome-wide meta-regression as implemented in MR-MEGA.

Results: Genomic inflation was negligible: lambda = 1.05 and LD score regression intercept = 1.04. Across ethnicities, we identified 308 1-Mb segments containing ≥1 SNP associated with eGFR (p value ≤ 5E-08): 228 of these loci were novel and 80 validated previously identified signals. Trans-ethnic meta-regression revealed limited

heterogeneity correlated with ancestry for most genomic regions. Approximate conditional analysis of the European-ancestry dataset identified 269 independent variants, which together doubled the eGFR variance explained compared to previous estimates.

Conclusions: This study represents the largest screen of kidney function genetic loci to date. The large number of identified loci will contribute to increase our understanding of kidney function's biology.

C. Pattaro: None.

P18.35C
Multi-phenotype epigenome-wide association analysis of fasting glucose and insulin in 981 Finnish individuals

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Background: Multi-phenotype genome-wide association studies (MP-GWAS) of correlated traits have greater power to detect genotype–phenotype associations than single-trait GWAS. In our previously-developed MP-GWAS method, implemented in the SCOPA software, single-nucleotide polymorphism (SNP) genotype dosage is 'reversely' regressed on a linear combination of phenotypes. Considering the epidemiologic correlations between fasting plasma glucose (FG) and fasting insulin (FI) levels, we aimed to use a SCOPA adaptation for multi-phenotype epigenome-wide association analysis (MP-EWAS) for these two traits in non-diabetic individuals.

Methods: We developed the "methylSCOPA" software, extending the SCOPA approach to MP-EWAS using DNA [hyper/hypo]methylation data, and applied it to FG, FI and Illumina Infinium HumanMethylation450K BeadChip array data from the Northern Finland Birth Cohorts (NFBC) 1966/1986. We subjected all data to stringent quality control and corrected them for measured (potential) confounders by linear regression analysis, and normalized the methylation signal intensity and FI data. The MP-EWAS included data for 635/346 individuals and 459,378/466,290 methylation probes from NFBC1966 and NFBC1986, respectively. We meta-analyzed the study-specific MP-EWAS results, mapped genomic locations to CGCh37/hg19, and adopted $p<1\times10^{-7}$ to denote epigenome-wide significance.

Results: In meta-analysis, the MP-EWAS association involving a methylation marker located at chromosome 22:49,088,813 and annotated to *FAM19A5* attained the lowest p-value ($p=1.4\times10^{-7}$) epigenome-wide. In single-trait FG and FI EWAS meta-analyses, the associations involving this marker attained $p=0.13$ and $p=4.0\times10^{-3}$, respectively.

Conclusion: We extended our MP approach to MP-EWAS, and demonstrated its enhanced power over single-trait analysis for detection of MP epigenetic effects in large-scale data.

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P18.36D
Characterisation and somatic mosaicism of the human glycophorin DUP4 structural variant, and association with hemoglobin levels in a malaria-endemic village

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Human glycophorins A and B are proteins expressed on the surface of erythrocytes, and are receptors for invasion of the *Plasmodium falciparum* parasite, which causes malaria in sub-Saharan Africa. The proteins are encoded by the genes *GYPA* and *GYPB* which, together with *GYPE*, reside on a tandemly-duplicated repeat region on chromosome 4q31.21. Previous genome-wide analysis has shown that a structural variant within this region (DUP4), is identical to the blood group antigen Dantu NE+, and confers a clinically-important protective effect, is common in East Africans and is strongly protective against severe malaria, reducing the risk of severe malaria by up to 40%. DUP4 is a complex structural genomic variant that carries hybrid (*GYPA*/*GYPB*) fusion genes. Using fibre-FISH, we validate the structural arrangement of the glycophorin locus in the DUP4 variant, and provide evidence of somatic variation in the number of *GYPA*/*GYPB* fusion genes. Subsequently, we have developed a paralogue-specific junction fragment PCR to genotype DUP4. We demonstrate association of DUP4 variant with hemoglobin levels - a phenotype related to malaria - in 348 unrelated DNA samples from a Tanzanian village holoendemic for malaria using a family-based association test. Using the family-based association approach implemented in (QTDT), we have found a statistically significant association of the DUP4 variant with

hemoglobin levels ($p = 0.0054$). Our study confirms the importance of the DUP4 variant in malaria protection, and raises the intriguing possibility of heightened somatic instability and somatic mosaicism at this locus in DUP4 carriers, which might confer added protection against malaria.

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P18.37A

Both common and rare genetic variants of *ABCG2* are risks for gout

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Introduction: Previous studies unveiled the association between gout susceptibility and common dysfunctional variants in *ABCG2*/*BCRP*, including rs72552713 (Q126X) and rs2231142 (Q141K). However, the effects of rare *ABCG2* variants on gout is unknown. Therefore, we investigated their effects on gout susceptibility.

Materials and Methods: We sequenced all exons of *ABCG2* in 480 gout patients and 480 healthy controls (Japanese males). We also performed functional assay of non-synonymous ABCG2 variants and analyzed the correlation between urate transport function and scores from the protein prediction algorithms (SIFT and PolyPhen-2). Stratified association analyses and multivariate logistic regression analysis were used to evaluate the effects of *ABCG2* variants on gout susceptibility.

Results: We genotyped 3 common and 19 rare non-synonymous variants of *ABCG2*. SIFT scores were significantly correlated with the urate transport function. After the effects of common variants were removed by stratification, the rare *ABCG2* variants were significantly associated with gout susceptibility (odds ratio [OR]=3.2, $p = 6.4\times10^{-3}$). Additionally, multivariate logistic regression analysis clarified that these rare *ABCG2* variants (OR=2.7, $p = 3.0\times10^{-3}$) were similar in effect size to Q126X (OR=3.4, $p = 3.2\times10^{-6}$) and Q141K (OR=2.3, $p = 2.7\times10^{-16}$).

Conclusions: We showed that both common and rare variants of *ABCG2* are independent risks for gout. These results could support not only "Common Disease, Common Variant" but also "Common Disease, Multiple Rare Variant" hypotheses for the association between *ABCG2* and gout.

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P18.38B

Genome-wide association study on the complement system activation by the classical, lectin, and alternative pathways in the Cooperative Health Research in South Tyrol (CHRIS) study

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The complement system (CS), a fundamental component of the innate immune response, can be activated via the classical (CP), lectin (LP), and alternative (AP) pathways. We conducted a genome-wide association study (GWAS) of the three pathways in a general population framework. CS activation was assessed in 4990 participants of the CHRIS study, using the WIESLAB commercial assay. For each pathway, GWAS were performed on >7 million dosage levels, imputed on the Haplotype Reference Consortium dataset, and selected for minor allele frequency of ≥0.01 and imputation quality of ≥0.3. Association models accounted for sex, age, plate and date of experiment, and relatedness. For significant SNPs, SNP-by-sex interaction was tested via linear mixed models. SNP-based heritability was estimated using GCTA-GREML. All SNPs explained 14%, 13%, and 45%, of the CP, AP, and LP variance, respectively. We identified associations at 8 loci carrying 12 independent variants with large effects: for AP, at chromosomes 1q31 ($p = 8.8e-10$), 5p13.1 ($p = 1.8e-17$), 6p22.1 ($p = 4.7e-14$), and 12p12.2 ($p = 1.8e-9$); for CP, at chromosome 6p22.1 ($p = 1.4e-35$); and for LP, at chromosomes 1p36.22 ($p = 7.3e-11$), 9q34.2 ($p = 7.6e-45$), and 10q21.1 ($p = 1.4e-260$). Six of these loci carry genes known for their relation with CS activation, as partially confirmed by summary-data-based Mendelian randomization analysis. SNP-by-sex interaction analysis identified a possible interaction between the 1p36.22 SNP and sex in association with LP ($p = 0.023$). We identified eight loci, most of which carry genes known to be involved in CS activation. Further characterization is warranted to define their pleiotropic landscape and involve CS activation in complex disease etiology.

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P18.40D

Genome-Wide Association Study of Serum Uric Acid in Korean

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Elevated serum uric acid (SUA) contributes to diverse health outcomes including gout, hypertension, diabetes mellitus and cardiovascular disease. The incidence and prevalence of hyperuricemia have been increasing. The proportion of heritability contributing to SUA levels is estimated to be about 40~70%. We aimed to identify genetic variants involved in the regulation of SUA using genome-wide association study (GWAS) in Korean. Genetic association was assessed with 4,414,664 single nucleotide polymorphisms (SNPs) after genomic imputation in 1905 unrelated men. We identified four loci (FRMD8, OVOL1, ABCG2 and NRXN2) that reached genome-wide significance ($<p<5\times10^{-8}$). The strongest association was found with a SNP in FRMD8 (rs184521656, $p = 3.02\times10^{-18}$), which has not been reported to be associated with SUA in the other studies. The other two loci (ABCG2 and NRXN2) were frequently reported in previous studies of several ethnic groups. In addition, we conducted replication study of the SNP, rs184521656 in 2912 Koreans including both men and women. Rs184521656 ($p = 1.50\times10^{-10}$) was successfully validated. Rs184521656 is found only in East Asian with a minor allele frequency (MAF) of 0.01 and functions as a protective allele in the direction of lowering the SUA in our study. This study validated previously published results and suggested new possibilities.

S. Im: None.

P18.42B

A retrospective analysis of the prevalence of imprinting disorders in Estonia: updated results

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Introduction: Imprinting disorders (IDs) are a group of rare congenital diseases affecting growth, development, metabolism and behaviour. Because of high clinical and genetic heterogeneity, the exact prevalence of IDs is not known.

Methods: In this study we retrospectively reviewed records of all Estonian patients with both molecularly and clinically diagnosed IDs found during the period 1998-2017.

Results: A total of 83 individuals with IDs were identified. 31% (26) of them had Prader-Willi syndrome (PWS), 18% (15) Silver-Russell syndrome (SRS), 17% (14) Angelman syndrome (AS), 14,5% (12) Beckwith-Wiedemann syndrome (BWS), 11% (9) pseudo- or pseudopseudohypoparathyroidism (PHP/PPHP), 5% (4) central precocious puberty (CPP), 2,5% (2) Temple syndrome (TS14) and 1% (1) transient neonatal diabetes mellitus (TNDM). 1/3 of all SRS and BWS cases fulfilled diagnostic criteria for these disorders, but were negative for genetic abnormalities. Age at diagnosis varied from prenatal to 83 years. During the period 2004-2017 birth prevalence of PWS in Estonia was 1/14,565 live births (1/10,000-1/25,000 worldwide), AS 1/29,130 (1/12,000-1/20,000), BWS 1/22,656 (~1/15,000), SRS 1/16,992 (1/75,000-1/100,000) and PHP/PPHP 1/33,985 (prevalence unknown). The total prevalence of IDs in Estonia is 6.3/100,000.

Conclusions: The birth prevalence of PWS has been found to be as expected, but the prevalence of AS and BWS was ~1.5 times lower. Probably the real worldwide prevalence of SRS is underestimated and may be at least 4 times higher than expected. The GNAS-gene-related IDs (PHP/PPHP) may also be relatively frequent disorders. This work was supported by the Estonian Research Council grant PUT355.

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P18.43C

Effects of inbreeding on linkage disequilibrium for SNPs of MTHFR, MTR, F5, LCT and VDR3 genes in Ukrainian population

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Introduction: The trend to assortative marriages in European countries leads to population subdivision and increased inbreeding level. It results in decreased population diversity, loci similarity, intensified recombination processes associated with chromosome rearrangements. The study was aimed to assess the gene linkage extent at different exogamy degrees of subjects.

Materials and Methods: The genotyping was carried for MTHFR 677C-T, 1298A-C, 1p36.3 (n=105), MTR 2756A-G, 1q43, F5 1691G-A, 1q24.2 (n=50), LCT C/T(-13910), G/A(-22018), 2q21.3 (n=34), VDR3 61888G>T, 61968T>C, 12q13.11 (n=100). The chromosome structure was analyzed using classical cytogenetics methods, GTG, FISH (n=6156). Exogamy degree scale: from 1 - parentage originated from one settlement to 4 - interethnic marriage. Inbreeding level was measured with F_{ST}. The linkage disequilibrium (LD) was estimated by D', r^2.

Results: F_{ST} in urban and rural Ukrainian populations in 2016 were 81.6×10^{-6} and 124.1×10^{-6}, increased over past 30 years up to 4 times. D' (r^2) for SNPs analyzed were: 1p36.3 - 0.045 (0.010), 1q43/1q24.2 - 0.025 (0.010), 2q21.3 - 0.209 (0.554), 12q13.11 - 0.039 (0.006). Increased exogamy degree was associated with increased D' (r^2) in loci 1p36.3: 0.027 - 0.081 (0.003 - 0.025), 1q43/1q24.2: 0.015 - 0.027 (0.002 - 0.004) and 12q13.11: 0.043 - 0.073 (0.008 - 0.023). Among all detected balanced translocations (n=69, 1.12%), 24.6% were in: 1p36.3 (2.9%), 1q43 (5.8%), 1q24.2 (4.4%), 2q21.3 (10.1%), 12q13.11 (1.4%). There were no translocations in other loci of chromosomes analyzed.

Conclusions: Considering pleiotropic effects of genes studied, increased F_{ST} leads to multifactorial diseases. LD could be used as indirect inbreeding evaluation.

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P18.44D

Linkage disequilibrium maps in sub-Saharan African populations

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Identifying patterns of linkage disequilibrium (LD) is of paramount importance for effective application of GWAS in mapping human complex traits. The LD structure in each population reflects the historical impact of recombination (along with other evolutionary forces) that shapes the current human population. Using the Malécot-Morton model we constructed LD maps across six sub-Saharan African (SSA) populations using the LDMAP program. The LD maps are constructed on the scale of linkage disequilibrium units (LDU) in which the decline of LD to 'background' levels across a variable physical distance corresponds to one LDU. Our results demonstrate that although population-specific LDU-maps present highly concordant contours, the magnitude of LDU steps differs across populations and is reflective of the duration since the last effective bottleneck. Furthermore, regions of the genome that have been influenced by various effective evolutionary forces such as mutation, selection or drift may define the remaining differences between population-specific LDU maps. Cross-population comparison of LD maps suggests that recombination hotspots are localised in all populations and can be leveraged in models of gene essentiality for disease gene prediction. We posited here that recombination rate and haplotype diversity can be considered as a proxy for gene essentiality and establish the interesting relationship between gene function and LD strength. The weaker LD in African populations achieves unprecedented resolution for functional analysis of LD maps and provides a basis for developing integrated models of gene essentiality and pathogenicity in the context of human disorders.

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P18.45A

Shared genetic etiology obesity with Barrett esophagus and esophageal adenocarcinoma: Insights from large genome-wide association studies

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Barrett esophagus (BE) is a precancerous condition of esophageal adenocarcinoma (EA), characterized by the replacement of normal squamous epithelium by metaplastic columnar epithelium in the distal esophagus. BE and EA are multifactorial disorders. The aim of the present study was to elucidate the overall shared genetic etiology between BE/EA and obesity, one of the major risk factors. We applied cross-trait LD score regression (LDSC) to summary level results of our recently published BE/EA GWAS meta-analysis and the GIANT consortium. For GIANT data, we extracted genetic data for BMI, as a proxy for general obesity, and waist-hip-ratio (WHR), as a proxy for abdominal obesity. LDSRs were performed both sex-specific and sex-combined. We also performed analyses on single marker level and compared risk alleles for all genome-wide significant SNPs in the BMI and WHR studies with those reported in BE/EA meta-analysis.

LDSR in the sex-combined analysis revealed a genetic correlation (r_g) of 0.13 (standard error, se=0.04; $p = 2\times10^{-4}$) between BE/EA and BMI and 0.12 (se=0.05, $p = 0.01$) between BE/EA and WHR. The sex-specific analysis revealed a stronger correlation with BMI in women, whereas the correlation with WHR was stronger in men. Analysis on single risk marker level revealed a statistical significant enrichment of BMI/WHR associated variants in BE/EA GWAS data (P=0.0086).

Our results provide evidence that the WHR in men and the BMI in women are genetically correlated with BE/EA. Our data point towards sex-specific mechanisms by which obesity mediates the risk for developing BE/EA. Further research into elucidation of these mechanisms is required.

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P18.46B

Genetic footprints and functional analysis of polymorphisms in the *PKLR* gene

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PKLR encodes for pyruvate kinase, a red blood cell enzyme that catalyzes ATP in glycolysis. This gene is under selective pressure by Plasmodium in Africa. Mutations for PK deficiency have a dual role: association with resistance to malaria and susceptibility to *Salmonella typhimurium*. We hypothesized that malaria selective pressure in Africa shaped *PKLR* resistant genotypes in humans and those could be an evolutionary trade off for intracellular pathogens. Thus, aiming to investigate the *PKLR* polymorphisms

and its association to mycobacterial pathogens, we performed two independent case-control studies with leprosy and tuberculosis in Rio de Janeiro and Mozambique. Our results highlighted G allele of the four tested SNPs and the haplotype G/G/G/G as associated with leprosy susceptibility in individuals from Rio de Janeiro (haplotype frequency in cases – 0.39 – and controls – 0.27). Subsequently, the association was confirmed in a Mozambican TB case-control study. The frequency of the haplotype G/G/G/G was higher in cases (0.48) than in controls (0.38), suggesting the same direction of association in Mozambique. Interestingly, the frequency of the susceptibility haplotype was augmented in African-ancestry cohorts from EPIGEN, Salvador (0.56), and 1000Genomes, YRI (0.87), compared to European-ancestry cohorts, Pelotas (0.32) and CEU (0.27). In addition, GG-genotype was correlated to higher ferritin and haptoglobin loads in healthy subjects and leprosy patients, which supports a relevance role of the *PKLR* gene with mycobacterial infections. Finally, we evidenced a locus of evolutionary trade off in which individuals carrying resistant selected genotypes to malaria infection were more susceptible to intracellular pathogens.

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P18.47C

Fine-scale linkage disequilibrium structure of functional elements within genic and sub-genic sequences

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Introduction: Next-generation sequencing (NGS) technologies have become high-throughput methods to identify disease-causing variations. However, NGS involves sequencing of millions of DNA pieces simultaneously leading to big data analyses, which may detect hundreds or thousands of apparently deleterious, but false positive, variants per sample. The integration of gene-specific properties, including patterns of linkage disequilibrium (LD), may help prioritise genes most likely to be involved in disease and reduce the number of the false positives. The aim of this study is to analyse LD maps of the autosomal genome at a very fine scale, down to the exonic and intronic level, providing novel insights into the impact of recombination and selection on genome structure and function.

Materials and Methods: We analysed single nucleotide polymorphism data from whole genome sequence data for 597 individuals from the Wellderly study. LD distances were computed according to the Malecot-Morton model which defines LD maps in LD units (LDUs).

Results: The results suggest that LDU/cM ratios of chromosome lengths are nearly constant in human populations, where the recombination is the primary determinant of LD structure. LD is more extensive in genic than intergenic regions demonstrating raised natural selection and decreased recombination in genes. The overall difference in the extent of LD between exons and introns is small but LD is more extensive in exons.

Conclusions: LD structure contains important insights into genome function to improve understanding of disease-causing variation. Therefore, gene-specific LD structure may be useful to improve filtering of NGS variant lists of disease candidates.

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P18.48D

Predicting rare allele carriers from genotyping-array data using whole genome sequencing data in the Estonian population

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Introduction: Genetic imputation works well with frequent alleles (minor allele frequency>1%), however, its predictive accuracy drops for rare genetic variants which can also play a role for eventual development of diseases. Exceptions to such limitations are endogamous populations like Estonia. Currently, 50,000 participants of Estonian biobank have been genotyped, with additional 100,000 being collected within next year, and their genetic profiles will be added to electronic health records. We performed a study to predict and verify rare mutation carriers for severe disease predispositions such as familial breast cancer (in genes *BRCA1* and *BRCA2*) and familial hypercholesterolemia (*APOB*) using long range haplotyping (LRH) and "surrogate parent" theory.

Materials and Methods: We used whole genome sequence data of 2,244 and genotyped data of 15,416 participants of the Estonian biobank. Whole genome sequencing (WGS) with 30x coverage was carried out at Broad Institute using Illumina HiSeq xTen platform.

Results: WGS identified 14, 4 and 6 mutations carriers for *BRCA1*, *BRCA2* and *APOB*, respectively. We identified 16 mutations carriers for *BRCA1* and 3 for *BRCA2*, and 5 carriers for *APOB* using LRH among genotyped samples.

We failed to find carriers for additional 6 out of 9 mutations, highlighting these as recent mutations and only present in limited historical lineages.

Conclusion: LRH is a cost-effective approach to predict additional rare mutation carriers for different disease predispositions from genotyped data in endogamous populations and will be important in the process of reporting clinically relevant mutations to Estonian biobank participants.

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P18.49A

Mosaic loss of chromosome Y (LOY) in blood helps explain why men live shorter lives

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Introduction: A growing number of papers shows that LOY in blood cells is associated with increased risk for all-cause mortality and disorders such as cancer, Alzheimer's disease, and cardiovascular disease. Known risk factors to be affected with LOY include age, smoking and inherited genetic variants. How much of the increased mortality of men is associated with LOY? How can losing chromosome Y in blood cells increase risk for disease in other organs?

Materials and Methods: To answer these questions we are studying functional consequences of LOY at DNA-, RNA- and protein-levels. LOY-induced transcriptional dysregulation are estimated in single cells using 10X Chromium system and changes in plasma proteins by Olinks proximity extension assays (PEA). FACS sorting are used to compare levels of LOY in different immune cells in men with cancer, Alzheimer's and controls.

Results: Our data suggests that the mechanism(s) behind associations between LOY and a various diseases could be related to disrupted immune cell functions in cells with LOY. Furthermore, men live on average about 6 years shorter compared to women and new analyses of epidemiological data shows that LOY explains up to 70% of the increased mortality.

Conclusions: The causality still remain an open question but new data suggest that disrupted immune system function(s) in cells with LOY could mediate reduced protection from different disease processes. LOY in blood could become a predictive biomarker for increased risk of common disease in middle-aged and aging men.

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P18.50B

Genotype-phenotype correlation inMarfan spectrum: application of NGS to study the role of rare and common genetic variants

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Introduction: Diagnosis of Marfan Spectrum is based several clinical criteria. Although identification of pathogenic variants contributes to the diagnostic process, its value for the prediction of clinical outcomes is still limited. The aim of the study was to extend the genotype-phenotype correlation starting from genetic data obtained by NGS analysis.

Materials and Methods: A cohort of 181 patients were analyzed. NGS panel included 10 known causative genes for Marfan spectrum (FBN1, TGFBR1-2, COL1A1-A2, COL3A1, COL5A1-A2, MYH11, ACTA2). An algorithm was developed to obtain a quality control checked pedigree file containing genotypes. Rare and common variants association analysis was performed using the appropriate statistical method and results were adjusted for multiple testing.

Results: More than 62% genetic variants were rare (MAF<0.01). The overall number of rare variants was significantly associated with the number of clinical manifestations ($p < 0.008$). Four common variants in the COL1A1 were significantly associated with skeletal outcomes ($p< 0.05$). Both rare and common variants were found to significantly contribute to clinical spectrum.

Conclusions: The genotype-phenotype correlation was extended to the overall common and rare genetic variability observed in NGS data. This approach increased the percentage of phenotypic variability explained by genetic factors.

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P18.51C

Mendelian Randomization Analysis to Explore a Causal Relation between Triglyceride Levels and HbA1c Levels

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The relationships between triglyceride (TG) and elevated glucose /HbA1c/type 2 diabetes (T2D) have been reported in many studies. The causal relationship is ambiguous. We used Mendelian randomization analysis to explore a causal relation between elevated TG leading to elevated HbA1c in 16,000 participants of Taiwan Biobank Cohort. TG showed a high correlation in T2D and hyperlipidemia, it was generate a hypothesis that TG may play an important role in T2D and HbA1c. We selected 7 SNPs and a genetic risk score (GRS) composed of these 7 SNPs, which were associated with TG specifically in East Asian populations. The intercept of MR-egger regression was with a confidence interval including the null (intercept, 0.143; 95% CI -0.868-1.154; P=0.78), suggesting that pleiotropic effect did not influence the result. Furthermore, the GRS was not significantly associated with confounding factors. Based on GRS, the association between TG and HbA1c shows that one standard-deviation increase in TG-associated GRS was significantly associated with a 9% increase in odds of being high HbA1c (>6.8%) (OR: 1.09, 95% CI: 1.001-1.196, P=0.048). The finding suggests a causal role of high TG level leading to HbA1c. To confirm our results, we performed bidirectional MR analysis. It show that the genetic determinants of HbA1c don't contribute to TG, suggested by a non-significant beta value. (beta:1.748, 95% CI:-11.497-14.9), and indicating no causal role of HbA1c level to affect TG level. The present study supports a causal relationship of high elevated TG resulting in high HbA1c, and, by inference, the development of T2D.

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P18.52D

Genetic risk variants of ZPR1/BuD13 for metabolic syndrome and metabolic components identified in Tehran Cardio-metabolic Genetics Study (TCGS)

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Introduction: Metabolic syndrome (MetS) plays the main role as one of the risk factors of cardiovascular disease. Some studies demonstrated that 11q23-25 region played a potential role in the pathogenesis of MetS. This study was performed to estimate the association of 20 tagged SNPs in BUD13/ZPR1 cluster with MetS components on Tehran Cardio-metabolic Genetics Study (TCGS).

Material and Methods: All participants (10,635 unrelated) were selected from the TCGS (male 46.2%) with the average age of 41.29±19.1 years which classified by JIS definition. DNA samples were genotyped with HumanOmniExpress-24-v1-0 bead chips. SNPs were set according to their LD, categorized thru Haploview software and their association was tested via kernel association test (SKAT). For the purpose of single analysis, linear regression analysis was used under recessive inheritance model after confounders adjustment.

Results: The result of SKAT model among 3 SNPs sets with the size of 5, 1 and 12 shown the last set in 3.3 kb has significant association after multiple testing corrections (FDR=0.0002). The result of single SNP association analysis has shown that TG has the most significant associated signals in this region and the remarkable lowest p-values belonged to two variants of ZPR1 (S.E±ß;p-value, rs2266788 "5.5±57.2;8.84E-25" and rs651821 "6.3 ±55.6;1.91E-18").

Conclusions: This study indicates the notable association of ZPR1 SNPs with TG blood concentration among the Iranian population. Although most investigations of lipid profile associated with chromosome 11 belongs to Apo A cluster, this study suggests that neighborhood regions are very likely to have significant effects on TG metabolism.

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P18.53A

A metabolomic comparison between the ischaemic strokes and coronary artery diseases: A Mendelian Randomization approach

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Dyslipidemia is well-established, and one of the strongest risk factors of two major cardiovascular diseases - ischaemic strokes (IS) and coronary artery diseases (CAD). Although IS and CAD share many risk factors, differences are also evident; A direct comparisons between IS and CAD has been difficult despite its potential value.

An NMR-based metabolomics analysis was performed for 1,000 Korean adult individuals (with ~ 230 metabolites) with full genetic, epidemiologic and clinical information. We performed a genome-wide scan for all metabolomics traits, and compared the results with existing compatible metabolomics-genomics analysis. We systematically identified IVs of each metabolite using following standards: 1) SNPs which was previous reported to have genome-wide significance with metabolite 2) markers among 1), which were replicated ($p<0.01$) in the Korean studies. We examined metabolites' genetic IVs for their roles in the IS (Metastroke) with CCS subtypes, and also in CAD (using GWAS results (CardiOgramPlusC4D). The associations were analyzed using different threshold p-values.

Among 230 metabolites, 140 overlapped with previous metabolomics-genomics studies and were included. A total of 114 metabolites, mainly those related to lipid metabolisms were associated either or IS and CAD at $p = 0.01$ level. Notably, all the metabolites causally associated with IS showed associations with CAD too. Some of the metabolites showed associations specifically with CAD (ApoA1/ Esterified.Cholesterol/ Fatty Acid Len/ Fatty Acid (FA) Omega-3, Medium-LDL Phospholipd (PL) / Mono Unsaturated FA/ Sphingomyelin /Extrasmall VLDL Particles/ Extrasmall Extrasmall VLDL triglyceride, extremely large VLDL Lipids, extremely large VLDL PL, extremely large VLDL particles, extremely large VLDL Triglyceride)

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P18.54B

Neurexin (*NRXN2*) and other components of the synaptic vesicle machinery: the importance of gene-gene interaction in migraine susceptibility

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Introduction: Migraine is a common neurological disorder affecting about 15% of the general population. In the last years, we have centered our attention to the synaptic vesicles' molecular machinery and life cycle, with a central role in neurotransmitter release and its regulation.Neurexin (*NRXN2*), a component of the synaptic vesicle machinery, forms connections between the fusion proteins of intracellular and synaptic vesicles, interacting with other important components of this mechanism as synaptotagmin, *$GABA_A$-R* or *CASK*.

Objective: Our aim is to further explore the role and interaction of these proteins involved in the regulatory mechanisms of neurotransmitter release, in migraine susceptibility.

Methods: Four tagging single nucleotide polymorphisms (SNPs) of *NRXN2* were analyzed in 183 cases and 265 controls. To evaluate association between *NRXN2* SNPs and migraine, a multivariable-logistic regression was performed. Allelic and haplotypic frequencies were estimated. Interaction between *NRXN2-SYT*, *NRXN2-GABRE* and *NRXN2-CASK* was assessed by a multivariable-logistic regression and confirmed by a multifactor dimensionality reduction analysis.

Results: We found two strong and significant synergistic interactions between migraine liability and the following gene pairs: *NRXN2-GABRE* and *NRXN2-CASK* that remained significant after 1000-fold permutation-based correction.

Conclusions: For the first time a genetic interaction was found among *NRXN2*, one of *$GABA_A$*-receptors and *CASK genes* showing a synergetic effect of interaction between these genes in migraine susceptibility.

These genes interactions may be a small part of a higher network of genes, allowing us to better understand migraine etiology and leading to the development of new therapeutic approaches.

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P18.56D

A study of Kibbutzim in Israel reveals risk factors for cardiometabolic traits and subtle population structure

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Genetic studies in isolated populations have provided increased power for identifying loci associated with complex diseases and traits. We present here the Kibbutzim Family Study (KFS), initiated for investigating environmental and genetic determinants of cardiometabolic traits in extended Israeli families living in communes characterized by long-term social stability and homogeneous environment. Extensive information on cardiometabolic traits, as well as genome-wide genetic data, was collected on 901 individuals, making this study, to the best of our knowledge, the largest of its kind in Israel. We have thoroughly characterized the KFS genetic structure, observing that most participants were of Ashkenazi Jewish (AJ) origin, and confirming a recent severe bottleneck in their recent history (point estimates: effective size ≈450 individuals, 23 generations ago). Focusing on genetic variants enriched in KFS compared with non-Finnish Europeans, we demonstrated that AJ-specific variants are largely involved in cancer-related pathways. Using linear mixed models, we conducted an association study of these enriched variants with 16 cardiometabolic traits. We found 24 variants to be significantly associated with cardiometabolic traits. The strongest association, which we also replicated, was between a variant upstream of the *MSRA* gene, ≈200-fold enriched in KFS, and weight ($P=3.6\cdot10^{-8}$). In summary, the KFS is a valuable resource for the study of the population genetics of Israel as well as the genetics of cardiometabolic traits in a homogeneous environment.

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P18.57A
Modern approaches to address missing data in multi-phenotype genome-wide association studies

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Multi-phenotype genome-wide association studies (MP-GWAS) play an important role in improving the power for locus discovery. However, joint analysis of multiple phenotypes increases the proportion of missingness, leading the standardly applied complete case (CC) analysis to become ineffiecient. We investigated the properties of single imputation (SI), multiple imputation (MI), k-Nearest Neighbour (k-NN), and Random Forest (RF) within the MP-GWAS framework, and compared them with the CC analysis using simulation studies.

We simulated genetic data for 5,000/50,000/500,000 individuals using Hapgen2, and highly (r=0.64) and moderately correlated (r=0.33) phenotypes (3/30/120) for these individuals in the statistical package R. We randomly chose common (minor allele frequency (MAF)> 5%), low-frequency (1%<MAF<5%) and rare (MAF<1%) variants to be associated with the simulated phenotypes. We considered 1/10/20/50% missingness under the three mechanisms: missing completely at random (MCAR), missing at random (MAR), and missing not at random (MNAR).

The resulting betas and standard errors (SEs) from the MP-GWAS after applying the selected methods were compared to the true values from full data analysis as well as to those from the CC analysis. These analyses showed that MI/KNN/RF perform the best, followed by SI, even under the scenario of MNAR, although SI/MI assume at least MAR. For the CC analysis, the performance worsened when the number of phenotypes was increased, while the other approaches were not influenced by the number of phenotypes nor differences in phenotype correlations or MAF.

In conclusion, we recommend MI/KNN/RF imputation approaches over the commonly applied CC analysis, especially KNN/RF under MNAR.

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P18.58B
A whole-genome sequencing study associates *GRAMD1B* with Multiple Sclerosis risk and disease activity

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Introduction: While the role of common genetic variants in multiple sclerosis (MS) has been elucidated, the contribution of rare variants remains unclear. We performed a whole-genome sequencing (WGS) study in an Italian family with four relatives with MS.

Materials: A 40x-10x coverage WGS was performed in 4 affected and 4 unaffected relatives on an Illumina® HiSeq 2000 instrument, and a bioinformatic pipeline used to filter and prioritize rare functional variants. We used HumanOmniexpress array and the Merlin software for linkage analyses. A target sequencing of *GRAMD1B* was applied in 91 probands of familial MS cases. *GRAMD1B* gene expression in rat cells and tissues and in human peripheral blood immune cells was evaluated by qRT-PCR, while the protein expression was evaluated in immune and brain cells and brain tissues by immunofluorescence.

Results: We identified a missense c.1801T>C (p. Ser601Pro) variant in *GRAMD1B* gene located at the linkage peak (LOD: 2.194). Target sequencing of the gene revealed additional rare missense and splice-site variants, 2 of which (rs755488531 and rs769527838) absent in 1000 Italian healthy controls, and an additional missense variant (rs199604534) in 4 MS Canadian families. Functional studies demonstrated that *GRAMD1B*, a gene with unknown function, is expressed by different CNS and peripheral immune cells. Notably, *GRAMD1B* was downregulated in vessel-associated astrocytes of active multiple sclerosis lesions in autopsied brains ($p<0.05$) and by inflammatory stimuli in primary astrocytes ($p = 0.007$) and peripheral monocytes ($p = 0.0002$).

Conclusions: These findings suggest a role of GRAMD1B in inflammation and disease pathophysiology and open new avenues of investigation.

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P18.59C
Genetic testing in the diagnostic workup of vascular anomalies

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Introduction: Differential diagnosis of vascular malformations can be difficult. The identification of somatic genetic causes for many has opened the door for systematic genetic testing as an aide in clinical assessment. We evaluated the usefulness of such testing.

Methods: Data was collected from genetic analyses performed on >1800 vascular anomaly tissues obtained during surgical treatments or as biopsies. This represented about 1000 individuals. Mutations were screened for using targeted NGS for high coverage to identify low frequency somatic mutations in heterogeneous samples. Clinical diagnosis was compared to the genetic results.

Results: A causative mutation was identified in > 45% of patients; sometimes it was a germline mutation. PIK3CA was the most frequently mutated: >80% of isolated and syndromic LMs, and 20% of VMs. Somatic TIE2 mutation was identified in 60% of VMs, and GNAQ or GNA11 mutation in 70% of SWS and CMs. The genetic result fit with the clinico-radiologic diagnosis in most cases (+/- 920/1000 = 92%), but resulted in revised diagnosis in others. Importantly, it was helpful for differential diagnosis of lesions of small size, deeper localization and unusual presentation. Series of lesions without any mutation were also identified. They often clinically differed from those with a mutation.

Conclusions: Genetic testing has become a valuable tool for management of vascular anomalies. It is useful for confirming and guiding towards correct diagnosis, for recognising rare entities, and for identifying persons with a familial risk. It also allows to identify unclassified entities, which need further clinico-genetic characterisation.

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P18.61A

Mitochondrial DNA haplogroups in Bulgarians with potential pharmacogenetic effect

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Introduction: Although mitochondrial DNA (mtDNA) variants that define haplogroups are generally considered as neutral, they may modify the impact of drugs, toxic environmental exposures or other factors. The possible linkage of mtDNA haplogroups with sensitivity to certain drugs and adverse drug reactions was established in several studies. The aim of our research was to determine the mtDNA haplogroups with pharmacogenetic effect found in the Bulgarian population.

Materials and Methods: The analyzed sample comprised 855 healthy unrelated Bulgarian subjects. Their mtDNA haplogroup classification was performed by sequencing of the control region of mtDNA in blood DNA samples, followed by genotyping of coding region markers for confirmation of haplogroup assignment.

Results: The Bulgarian mtDNA gene pool is overwhelmingly represented by Western Eurasian haplogroups (H, U, JT, W, X, V, I). The most common haplogroup H (41.9%) can lead to toxicity of highly active anti-retroviral therapy (HAART) with increased lipoatrophy. Haplogroup T, found in 10.6% of modern Bulgarians, may be protective against lipoatrophy in HAART, but it is associated with peripheral neuropathy in treatment with nucleoside reverse transcriptase inhibitors. The carriers of non-haplogroup H mtDNA lineages have better respond to riboflavin in migraine treatment. MtDNA haplogroup J (7.9%) shows linkage with cisplatin-induced hearing impairment.

Conclusion: The accumulation of knowledge about the prevalence of mtDNA pharmacogenetic variants including those that are haplogroup defining in healthy and in subjects with adverse drug reactions in different populations will improve mitochondrial pharmacogenomics - a lagging area in the improvement of drug therapy.

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P18.62B

Mutation spectrum of *PAH* gene in phenylketonuria patients from Georgia

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Introduction: Georgia is located in the Caucasus region of Eurasia and populated mostly by Georgians. Due to the remoteness of Georgians from the main routes of incursions and migrations, the territory of Georgia was the object of great demographic homogeneity. This allows us to assume the existence of a unique mutation spectrum in monogenic disease genes in this country.

Materials and Methods: DNA samples of 135 PKU patients from Georgia were analyzed for the presence of 25 common *PAH* gene mutations using allele-specific MLPA method. Calculations were made for 124 unrelated probands (248 chromosomes).

Results: *PAH* gene mutations were detected on 85.1% of chromosomes. At least one pathogenic allele was detected in 94.4% cases. The most common *PAH* gene mutation among patients from Georgia is P281L (33.5% of alleles). The following frequent variants are IVS10-11G>A (20.2%), R261* (7.7%), L48S (4.8%), R261Q (3.6%), R408W (3.2%), E390G (2.8%), IVS2+5G>C (2.4%), E280K (1.6%), R252W (1.2%), A300S (1.2%). Also 7 different pathogenic variants were found in single cases.

Conclusions: Due to the *PAH* gene mutation spectrum, we can conclude, that Georgians are genetically far from nations living in contiguous territories. This feature is characteristic of most of the ethnic groups living in the Caucasus - they are quite original and preserved their national identity. Nevertheless, the frequent mutation method that was originally created for patients from Russia is suitable for diagnosing patients with PKU from Georgia.

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P18.63C

Neurodegenerative disorders associated with polyglutamine expansions: an Italian study to investigate the prevalence of Intermediate polyQ alleles in healthy subjects

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Introduction: Polyglutamine (PolyQ) diseases are inherited neurodegenerative disorders, commonly manifesting in adulthood, and caused by CAG repeat expansions in the coding regions of the respective disease-genes. Alleles with CAG repeat number slightly below the pathological threshold are defined as intermediate alleles (IAs). IAs are associated with reduced penetrance and meiotic instability, and may be responsible for new diagnosis in subjects without family history. In Italy the most common polyQ diseases are Spinocerebellar Ataxia type 1-2-17 (SCA1-2-17) and Huntington disease (HD). We aimed to investigate the frequencies of IAs in HD, SCA 1-2-17 in healthy Italian subjects.

Materials and Methods: We enrolled 366 healthy Italian subjects (177M/189F, aged 19-84) without familiar history. All subjects were informed of the purpose of the study and gave their written consent for CAG repeat screening in SCA1-2-17 and HD genes, according to European guidelines EMQN. Genetic counseling was offered to the individuals with IAs.

Results: One SCA1-IA (35 CAG; 0.1%) and three SCA17-IAs (42-44 CAG; 0.4%) were found in 4 unrelated individuals. Two subjects carried a SCA2-IA with 31 CAG repeats (0.3%), and 16 subjects had HD-IAs associated with meiotic instability (27-35 CAG; 2.2%).

Conclusions: The allelic frequencies of IAs in SCA1-2-17 and HD diseases in Italian population were similar to those previously reported. SCA1-2-17 IAs may be associated with extremely late-onset symptomatology. Moreover, SCA2-IAs are recognized as risk factors for ALS susceptibility. The high frequency of HD-IAs may represent a reservoir for fully pathological expanded alleles.

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P18.64D

Non-invasive prenatal testing as a valuable source of population specific allelic frequencies

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Introduction: NIPT for common aneuploidies is one of the most rapidly adopted and relatively low-cost DNA tests. However, the information from the resulting data are not used up to their full potential. High throughput sequencing on pooled samples, each sample from different individual, is a strategy to identify genetic variability at a small fraction of the cost required to do population scale studies. Here we describe the possible re-use of the data generated during NIPT for genome scale population specific frequency determination of single nucleotide variants (SNV).

Materials and Methods: To evaluate the limitations of this approach, data generated by massively parallel low coverage whole-genome sequencing of plasma DNA of 1,548 pregnant women undergoing NIPT procedure was analysed. We used statistical methods, such as PCA, for visualization and comparison our data to other studies.

Results: We detected 6,622,893 (0.21% of the genome) distinct potential SNVs. Nearly 98% of the variants were found to be already known in dbSNP allowing verification of our results on several levels. Allelic frequency distributions of our population (Central Europe) were compared to the six ExAC populations placing our sample set most closely to the two European ExAC populations that is consistent with the geographical and genetic relationships between the compared populations.

Conclusion: Re-using NIPT data to determine population frequencies of small DNA variants can be a real low-cost alternative to costly population scale studies.

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P18.65A

Where Europe and Africa meet: human genomic history of the western Mediterranean

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Introduction: In the last few years, genome-wide (GW) studies have become essentials in the fields of human genetics and genomic anthropology. Here, we performed a GW screening of a key geographic area: the western Mediterranean. Its metapopulation, at the crossroads between Europe and Africa, is a target for the study of human evolutionary history across continents.

Materials and Methods: We have analyzed six populations from southern Iberia and Morocco. Altogether, 142 samples were genotyped on Illumina's Omni2.5 array. A systematic pipeline was performed to merge our dataset with previously reported GW information. Depending on the SNP density (1.8M and 84k markers), two resolution levels were stablished. By using bioinformatic tools, demographic histories, admixture dynamics and genetic structure of the Mediterranean populations was assessed, along with global/local ancestries, admixture time modelling and the magnitude of population relationships.

Results: Our data showed a latitudinal gradient from northern to southern Europe with respect to the African genomic contribution. African ancestral proportion in the southwestern end of Iberia differs twofold than that found in most Iberian populations. The analysis of the tract-length distribution showed the presence of very short African ancestry tracts in southern Iberia. This scenario would be reflecting ancient African gene flow to Europe through the Iberian Peninsula. Our findings are compatible with two temporally spaced migratory events.

Conclusions: Genomic analysis provide clues to understand the complex role of southern Iberia and northwestern Africa as transition areas of old, recurrent human migrations across their respective continents. *Financial support: Spanish Ministry MINECO (CGL2014-53985-R).*

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P18.66B

Fine-scale population structure in western France

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Characterising the genetic structure of human populations provides insight into demographical history and informs research on disease association studies, especially on rare recent variants which tend to be geographically clustered. In this study, we examine the fine-scale genetic structure of Brittany, Anjou, Poitou and Maine in western France.

We genotyped 2,500 individuals whose 4 grandparents were born within a small distance in western France on Affymettrix Axiom PMRA array plates. Principal Components are highly correlated with geographical coordinates of grandparents' birthplaces (p-value < 2e-16). Visualisation of single principal components' values (from PC1 to PC5) on the map reveals patterns of local genetic structure. Those patterns are confirmed when samples are assigned their most probable source populations from ADMIXTURE. Loire River and its tributaries, Erdre and Sèvre Nantaise, seem to be at the limits of observed genetic subpopulations. Using Identity By Descent sharing (IBDNe), we show that identified subgroups may have followed different trajectories of effective population size evolution in the last 25 generations.

We here report existence of a fine-scale structure across western France, with evidence of distinct demographic histories between subpopulations. These results support the need for a genetically matched panel of controls from France, to avoid confounding effects of fine-scale population structure.

We will subsequently verify and extend our findings methods based on haplotype structure and IBD, on an extended population of 5,700 individuals. Further study of demographic models will yield not only insight on population history, but also provide a null model for tests of selection.

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P18.67C

Association of PRDM16 and CtBP2 genes polymorphisms with lipid profile of adolescents

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Introduction: Brown adipose tissue (BAT) presence in adolescents and adults has raised many questions about its physiology and possible influence on human health. In healthy adults, presence of BAT was associated with lower glucose levels, total cholesterol and LDL and levels. The key regulator of BAT development is PR domain-containing protein 16 (PRDM16). It induces brown fat phenotype by interaction with PGC1α, PPARα, PPARγ, C/EBP and represses white adipose tissue specific genes through the association with C-terminal binding co-repressor proteins (CtBP1 and CtBP2). Our aim was to analyze the association of *PRDM16* gene (rs12409277) and *CtBP2* gene (rs1561589) polymorphisms with BMI, fasting glucose level and lipid profile of adolescents.

Material and Methods: Our study included 300 healthy school children, 146 boys (48.7%) and 154 girls (51.3%), 15 years of age. Genotypes for the selected polymorphisms were detected by the Real-time PCR method. Age, gender, height, weight, lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides) and fasting glucose were recorded.

Results: We did not find statistically significant association between rs12409277 and rs1561589 polymorphisms with BMI, fasting glucose and lipid profile of adolescents. We further analysed combine effect of the two SNPs. Statistical analysis has shown that carriers of CT genotype of rs12409277 polymorphism and GG genotype of rs1561589 polymorphism had significantly lower total cholesterol ($p = 0.001$) and LDL cholesterol ($p = 0.008$) level when compared to all other groups of genotypes.

Conclusion: Our study suggests that rs12409277 and rs1561589 polymorphism might have influence on total and LDL cholesterol levels in adolescents.

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P18.68D

Healthcare burden of rare diseases in Hong Kong - the use of ORPHA codes in an ICD-10 based healthcare administrative dataset

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The healthcare burden of rare diseases (RD) is important but difficult to estimate. Walker et al. (2017) made use of cross-referencing between ORPHA codes and ICD-10 in health administrative datasets to identify RD-related admissions in Western Australia. Such methodology was adopted in Hong Kong which has recently awakened to the needs of RD patients.

Methods: We extracted from the local public healthcare database admission records of all patients coded with one or more of the 1084 ICD-10 codes cross referenced with 467 ORPHA codes from 2005 to 2016. We further analyzed RD-related inpatient healthcare cost using a subset of patients admitted during 2015 -2016.

Results: A total of 546,673 admissions were identified, representing 3.2% of total admission in 2005-2016. About 109,000 patients were alive at the end of the study, representing 1.5% of the Hong Kong population. The most common RD category in the pediatric age group was 'rare developmental defects during embryogenesis'; whereas that amongst adults was 'rare haematological disease'. The aforementioned subset for 2015-2016 accounted for 330,091 patient-days, placing the estimated total inpatient cost for RD at HKD$1,594,339,530 (~EUR 163,612,000) i.e. 4.3% of total inpatient cost.

Conclusion: Cross referencing between ICD-10 and ORPHA codes may be adopted in different healthcare datasets for international comparison. Despite differences in the prevalence of individual diseases, the disparity between RD prevalence and associated inpatient cost is consistent across settings reflects the importance of RD in healthcare policies.

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P18.69A

Explaining RLS families using risk SNPs from GWAS

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Introduction: Restless legs syndrome (RLS) is as disease of the nervous system. At rest in the evening RLS patients experience an urge to move the legs which impacts sleep and quality of life. RLS has a strong family history. Here, we investigated whether RLS GWAS risk SNPs might explain RLS inheritance patterns in families.

Materials and Methods: Published RLS risk loci from GWAS were genotyped/imputed in 79 families of European descent (843 individuals) using the Affymetrix Axiom genotyping array. Logistic regression was applied to each single family for individual SNPs and polygenic risk scores (PRS), including the pedigree structure as a random effect to correct for relatedness in the association test. The model fit was quantified using Nagelkerke's R^2 confidence intervals and significances were assessed empirically.

Results: In family-wise meta-analyses across SNPs (Fisher's method), three families showed a significant association after correcting for multiple testing of families. In one family, two SNPs alone almost reached significance at the *MEIS* and *BTBD9* locus after correcting for multiple testing of SNPs and families. No family reached significant association using a PRS.

Conclusion: We hypothesize that risk SNPs might explain only some RLS inheritance patterns, either through tagging of a causal variant or interaction with other family-specific RLS triggering genetic/environmental factors.

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P18.70B
Interaction of environmental and genetic factors in school myopia development

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Since the formation of the compulsory education system for children worldwide many researchers observe an increase of myopia incidence in children attending schools, so called school myopia. Nowadays, the question of why the risk of myopia becomes high with the beginning of the school remains unanswered. On the one hand, we must search for an environmental risk factor of myopia changed by spending time at school. On the other hand, while all children visit the school, not all of them develop myopia, therefore some children are more genetically sensitive to the environmental factor we are looking for. So, if we find genetic factor of the myopia, and this factor is regulated by some environmental factor, then we can confirm this environmental factor to be a risk factor of myopia. In our work, we found out that the vitamin D receptor gene polymorphism A-3731G was associated with myopia in schoolchildren, and the carriers of (-3731A)-allele were more sensitive to the myopia. As the VDR gene signaling pathway is regulated by UV light we concluded that UV, or rather it's lack during the stay in the school building during the daytime, can cause the school myopia. This means that it is necessary to change hygienic norms of staying at school to prevent the development of myopia in children in the period of eye growth and vision formation. Because despite of the fact that correction of myopia is quite simple, myopia still worsens the quality of life and finally can lead to blindness.

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P18.71C
Utility of Slovene genomic variation database in diagnostic NGS

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Population specific genetic variability represents a cornerstone of interpretation of massive amounts of data generated by next-generation sequencing approaches. Large international sequencing initiatives have generated good coverage of genetic variation in major databases, but the utility of those is limited in distinct world populations. To tackle this issue we report our findings from clinical exome and whole exome sequencing of 1626 patients submitted to our institution and the implementation of locally gathered data for variant filtration in diagnostic NGS interpretation.

We selected functional variants both benign and pathogenic from the analyzed samples which yielded nearly 94 exonic non-synonymous variants per sample, of which over 43 variants per sample were considered rare, present only in a single individual in our database. Furthermore, aggregating detected CNVs, nonconsensus splice site variants, mitochondrial variants and genomic breakpoints provided additional information on distinct Slovenian genomic variability. In total, we discovered over 28 thousand

variants previously unreported in population variation databases, which also proved beneficial in the diagnostic process by having a positive effect on the overall workload of variant interpretation. Overall, our data provided us with 18,4% increased variant filtering power, significantly supplementing the sequencing data of Genome Aggregation Database (gnomAD).

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P18.73A

Violence exposure, stress biomarkers and gender differences on buccal telomere length in African American young adults

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Violence exposure has long-lasting social and biological impacts on African American (AA) health and can lead to physiological changes, including shorter telomere length (TL). While studies have investigated the relationship between TL and life stress, few focus on African American young adults and their direct nexus to violence. This study examines the effect of violence and gender on both stress biomarkers and TL in AA young adults. We examine the relationship between violence exposure, seven stress biomarkers (IgA/G/E/M, C Reactive Protein, Cortisol, Epstein Barr Virus Antigen) and TL in a cross sectional analysis of 50 buccal samples (N=25 males & 25 females) of AA 18-25 years old in Washington DC who experience differential violence exposure (physical, threat, witnessed, and sexual). Average TL was measured by qPCR. Mann-Whitney tests identified differences between males and females in exposure to violence, stress biomarkers, and the measures of TL. Correlations were calculated between TLs, biomarker levels, and violence measures. Elevated sexual violence exposure was positively correlated (R_{RANGE}= 0.22 to 0.55) with all elevated stress biomarkers except IgE. TL in the high violence exposure group was negatively correlated to all stress biomarker levels (R_{RANGE}= -0.09 to -0.31) except for IgG. There was no significant difference in TL among females exposed and not exposed to violence. Violence exposed females had a longer TL than men ($Mean_{F:M}$=1.87 v 1.62, p≤.054). Male sexual violence exposure was correlated to TL (R=0.575, p≤ 0.03). High violence levels correlate to shorter TLs and higher stress biomarker levels in AA young adults.

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P18.74B

Evolutionary rewiring of human regulatory networks by waves of genome expansion

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Introduction: Divergence at the regulatory level underlies a significant fraction of the phenotypic differences between extant species, with genome expansion potentially playing a key role in the evolution of gene regulation. Thus, we investigated how newly arising sequences contributed to the rewiring of regulatory networks in the human genome throughout its evolution from the common ancestor of all Vertebrates.

Methods: First, we estimated the evolutionary age of each region of the human genome by applying maximum parsimony to genome-wide alignments with 100 vertebrates; we then studied the age distribution of several types of functional regions, focusing on regulatory elements.

Results: The age distribution of regulatory elements revealed the extensive use of newly formed genomic sequence in the evolution of regulatory interactions: many transcription factors have expanded their repertoire of targets through waves of genomic expansion that can be traced to specific evolutionary events. Repetitive elements greatly contributed to such expansion: several classes of these elements are enriched in binding sites of one or a few specific transcription factors, with such binding sites being localized in particular portions of the element and characterized by distinctive motif words.

Conclusions: Taken together, these features suggest that the binding sites were available as soon as the new sequence entered the genome, rather than being created later by accumulation of point mutations. By comparing the age of regulatory regions to the evolutionary shift in expression of nearby genes, we show that rewiring through genome expansion played an important role in shaping current human regulatory networks.

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P18.75C

Identification of founder effect and haplotype reconstruction in transthyretin amyloidosis patients with Glu89Gln mutation in the *TTR* gene from endemic region in Bulgaria

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Familial transthyretin amyloidosis is an autosomal dominant genetic disorder caused by missense mutations in the *TTR* gene resulting in amyloid plaques formation of the transthyretin protein. Depending on the system affection the manifestations may be different and high heterogeneity in the penetrance is observed. An endemic region in Bulgaria exists where the *TTR* mutation Glu89Gln is found with high frequency. This is a rare mutation and was probably introduced in the population by a common ancestor. This phenomenon, called "founder effect" was proved in carrier families with haplotype analysis of microsatellite markers showing linkage disequilibrium. Allele frequencies were analyzed and haplotype reconstruction was done with *Arlequin v.3.01* software. The common ancestry of the carriers was demonstrated using additional data for their genealogies and microsatellite data from a control group of non-affected individuals. The results show that the mutation Glu89Gln is linked to one haplotype, called "hypothetical founder haplotype" which was compared to haplotipic data from other European patients and no similarity was found. The fact that the founder haplotype has been subjected to decay was used to determine the mutation age using *DMLE+ v.2.0* software. The results demonstrated that the mutation arouse 850 generations ago or 15 300 years, assuming average reproductive age of 18 years. The haplotype analysis of patients with transthyretin amyloidosis could help with differentiation of subgroups showing the same phenotype in order to investigate the heterogenic manifestation of the disorder. Acknowledgement: The study was supported by Pfizer: Grant №WI220557/15.11.2016.

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P18.76D

Association of immunogenetic factors with susceptibility to pulmonary tuberculosis in Sahariya tribe of North Madhya Pradesh

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Introduction: Sahariya, a primitive tribe of Central India, has shown an increased incidence of TB as compared to other tribes. It suggests the role of host immunogenetic factors for such a high incidence of PTB. Thus, in view of the unique ethnicity of Sahariya, the association of immunogenetic factors like Chemokines and HLA with PTB was determined.

Methods: About 400 individuals from Sahariya tribe were genotyped for *HLA* genes and SNPs in *Chemokine* using luminex based methods and ARMS-PCR respectively. Plasma level of positively associated chemokine was also determined using commercial ELISA kits. The data was statistically analyzed by using SPSSv16.0.

Results: The allelic frequencies of HLA-DRB1*15:01 and 15:02 were significantly increased in the PTB patients than in healthy controls (p=0.04). However, DRB1*16:02 was found to be significantly reduced in PTB patients (p=0.003). Further, the frequencies of 'AA' genotype and 'A' allele of -403G/A SNP of CCL5 gene were found significantly higher in cases than in controls which resulted in increased plasma CCL5 level. However, the level was decreased significantly in patients who were on therapy or have completed their therapy ($KWp = 0.04$).

Conclusions: Our study for the first time, showed a unique association of HLA-DRB1*16:02 with protection against pulmonary tuberculosis. Additionally, we found that the CCL5 level may not only be influenced with the genotypes of -403G>A SNP and bacillary load but also with the treatment. Thus, these genetic factors could be used as a diagnostic marker and could be useful in designing drug to target tuberculosis.

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P18.77A

Deletions at 63 GWAS catalog loci based on genome-wide 1000 Genomes project CNV-tagging SNPs

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Background: Genome-wide association studies (GWAS) successfully exploit the variability of most abundant DNA variants, namely single nucleotide polymorphisms (SNPs), but frequently fail to provide information about causal mutations. Copy number variation (CNV) impacts phenotype variability and disease susceptibility and is one of the sources for the so-called "missing heritability". Despite notable genomic effects of both CNVs and SNPs, the correlation between them is understudied, and the role of CNVs in SNP-based phenotypic effects is not established.

Methods: We estimated linkage disequilibrium (LD) between CNVs and SNPs in protein-coding genes using the 1000 Genomes project sequencing data (1000G) from phase 3. We defined CNV-tagging SNPs for variants reported in the GWAS catalog for disease/phenotype associations (July, 2017) and for recently published DIAGRAM consortium type 2 diabetes (T2D) 1000G reference panel-imputed meta-analysis in Europeans (PMID:28566273).

Results: We replicated established CNV-tagging SNP effects at ten loci, including *NEGR1, LCE3A/B, CFHR1-3* for obesity, psoriasis and nephropathy, respectively. We revealed 31 novel CNVs (length 275bp to ~ 6kb), all but one being deletions. Among novel CNVs fifteen are <1kb, tagging lead breast cancer and T2D/lupus erythematosus SNPs at *CHST9* and *JAZF1* loci among others. Novel CNVs covered drug-target genes, such as *HTR3D/C/E, PLEKHA1*, and *MGST1* and tagged SNPs associated with major depressive disorder, age related macular degeneration, and visceral fat, respectively.

Conclusion: This is the most detailed CNV-tagging SNPs catalog to date, which will help in dissecting the functional impact of SNP-trait associations and could drive the development of new drugs.

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P18.78B

Improvementof survival in vascular Ehlers-Danlos syndrome: effect of celiprolol in along-term cohort study

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Vascular Ehlers-Danlos syndrome (vEDS) is a rare genetic connective tissue disorder secondary to pathogenic variants within the *COL3A1* gene, resulting in exceptional arterial and organ fragility and premature death. No specific medical treatment has been proven as efficient in reducing the spontaneous arterial dissections/ruptures, but celiprolol, a specific beta-blocker that was shown beneficial in a limited open clinical trial. The main objective of this retrospective study was to analyze the outcomes of a large cohort of molecularly confirmed vEDS patients followed up to 17 years in a single referral centre, most of them being medically treated with celiprolol and scheduled for yearly follow-up. Between 2000 and 2017, 144 patients, (median age at diagnosis 34.5 years, 87 females, 91 probands) were eligible for this study. After a median follow-up of 5.3 years, overall patient survival was high (71.6%; 95% CI 0.5-0.9) and dependent on the type of *COL3A1* variant, age at diagnosis and treatment. More than two-thirds of patients remained asymptomatic, despite a large number of arterial lesions at the initial follow-up. Patients treated with celiprolol had improved survival ($p = 0.0004$) and the greatest protection was observed with 400mg/day compared to <400mg/day ($p = 0.003$). Hospitalization rates for symptomatic arterial events also decreased with the systematic use of celiprolol. In this long-term survey, vEDS patients exhibited a high survival rate compared to what was expected as well as a low annual occurrence of arterial complications. Despite the absence of a control group, celiprolol seems to have a significant positive influence on the rate of arterial complications and survival.

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P18.79C

Insights to the genetic architecture of quantitative traits in the Cooperative Health Research in South Tyrol (CHRIS) study

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When undertaking an association study of low-frequency and rare variants, there are compelling reasons to focus on variation in protein-coding sequence. First, coding variants are enriched for impact on molecular function and support more direct biological interpretation than associations within non-coding sequence. Second, the functional annotation of coding variants allows discovery efforts to benefit from the improved power offered by the aggregation of rare alleles presumed to exert broadly similar molecular effects. We sequenced the exomes of 3,549 individuals from the population-based CHRIS study and identified >389k variants. We then tested 58 quantitative cardiovascular and metabolic biochemical parameters for genetic associations. We performed single variant analyses using mixed-model approaches that account for relatedness (EMMAX). To increase power to detect rare variant effects, we performed gene-level tests using SKAT-O focusing on non-synonymous variants with minor allele frequency (MAF) < 0.05. We identified at genome-wide significance level 31 loci associated with at least one trait, of which 9 were not reported in the EBI GWAS catalog. The gene-level analysis revealed at gene-wide significance level 17 association, of which 10 were novel. These include novel association for kidney function, anticoagulation, and iron metabolism. Together, these results suggest the value of exome sequencing in well-characterized cohorts for gene discovery experiments.

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P19 Genetic counselling/Education/public services

P19.01A

A decision tree for the genetic diagnosis of deficiency of adenosine deaminase 2 (DADA2)

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Deficiency of adenosine deaminase 2 (DADA2) is a recently described autoinflammatory disorder. Genetic analysis is required to confirm the diagnosis. We aimed to describe the identifying symptoms and genotypes of patients referred to our reference centres and to improve the indications for genetic testing. DNA from 66 patients with clinically suspected DADA2 were sequenced by Sanger or next-generation sequencing. Detailed epidemiological, clinical and biological features were collected by use of a questionnaire and were compared between patients with and without genetic confirmation of DADA2. We identified 13 patients (19.6%) carrying recessively inherited mutations in ADA2 that were predicted to be deleterious. Eight patients were compound heterozygous for mutations. Seven mutations were novel (4 missense variants, 2 predicted to affect mRNA splicing and 1 frameshift). The mean age of the 13 patients with genetic confirmation was 12.7 years at disease onset and 20.8 years at diagnosis. Phenotypic manifestations included fever (85%), vasculitis (85%) and neurological disorders (54%). Features best associated with a confirmatory genotype included fever with neurologic or cutaneous attacks (odds ratio [OR] 10.71, $p = 0.003$ and OR 10.9, $p < 0.001$), fever alone (OR 8.1, $p = 0.01$), and elevated C-reactive protein (CRP) level with neurologic involvement (OR 6.63, $p = 0.017$). Our proposed decision tree may help improve obtaining genetic confirmation of DADA2 in the context of autoinflammatory symptoms. Prerequisites for quick and low-cost Sanger analysis include one typical cutaneous or neurological sign, one marker of

inflammation (fever or elevated CRP level), and recurrent or chronic attacks in adults.

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P19.02B
Delivering clinical bioinformatics education in the NGS era

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Development of next generation sequencing (NGS) technology has enabled panel, exome and whole genome sequencing for clinical diagnostics. Clinical NGS is becoming increasing commonplace. Large scale sequencing projects, such as the 100,000 Genomes project, are building capacity within the NHS, paving the way for whole genome sequencing of patients, and a more personalised approach to the delivery of patient care. The reduced cost of NGS enables more widespread testing. A recent UK study suggested all women over thirty should be offered sequencing of genes associated with breast cancer. Widespread integration of genomics into healthcare requires greater numbers of healthcare professionals with specialised data management and analysis skills, at a time when there is a bioinformatics skills shortage. Since 2013, the University of Manchester (UoM) has been delivering Masters programmes to educate bioinformaticians, clinical scientists and genetic counsellors working in the NHS. Close links between UoM and the Centre for Genomic Medicine, St Mary's Hospital Manchester enables us to ensure that training aligns with clinical practice and professional body standards. We recently trained 55 students in the use of the ACMG guidelines for variant classification and report here on how students utilised the guidelines to classify clinical variants. The skills shortage is a problem worldwide and we receive increasing interest from overseas students. In response, we developed a clinical bioinformatics MOOC (https://www.futurelearn.com/courses/bioinformatics), with over 11,000 participants to date. We are now developing a Distance -Learning Post-Graduate Certificate in Clinical Bioinformatics, with individual modules also available for CPD, launching in September 2018.

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P19.03C
Important lessons learned in the first year of mainstreaming BRCA testing at the north west thames regional genetics service

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The identification of targeted therapies for cancer has led to an increase in the demand of rapid gene test results within cancer MDT's. The Royal Marsden Hospital piloted Mainstreaming Cancer Genetics testing and this has now been rolled out by several Regional Genetics Services. The advantages of BRCA mainstreaming include fast, accurate testing within the cancer care pathway, enabling decisions about best treatments. In addition, it helps identify at risk relatives giving them the opportunity to decrease their cancer risk. We were keen to roll out this service and in 2016 following training to Gynaecological Oncologists in the North West Thames region, mainstream BRCA testing was offered for patients with non-mucinous ovarian cancer. The BRCA detection rate was 11.1% (7/63) and in addition two variants of uncertain significance (VUS) were detected. The overall experience has been positive. However the Gynaecological oncologists recognised an issue around informed consent as patients who had previously declined testing through Clinical Genetics then went ahead with testing. In addition patients given a normal BRCA result were disappointed when they were not eligible for certain treatments. Issues also arose around informing next of kin of results as some patients were terminally ill. The explanation of results such as lifetime risks of cancer and VUSs has been variable and a more streamlined pathway has been put in place. The benefits of this service have outweighed the drawbacks however interesting lessons have been learned which we felt were important to share.

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P19.04D
Mainstreaming BRCA1 and BRCA2 testing: an interview study of healthcare professionals' views

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The mainstreaming of genetic services in cancer care offers the promise of streamlined, tailored treatment for individuals, provided through simplified care pathways. We know little about how clinicians working outside clinical genetics view mainstreaming, particularly, how this impacts on their professional role and responsibilities. We conducted a qualitative study of healthcare providers at a UK teaching hospital, focusing on their views and experiences of providing treatment-focused *BRCA 1* and *BRCA 2* genetic testing (TFGT) for breast and ovarian cancer patients. Semi-structured interviews were undertaken with: two medical oncologists who offer TFGT to their ovarian cancer patients, six breast surgeons (and a breast care nurse specialist) who were responsible for triaging patients for onward referral to clinical genetics, six medical oncologists who were about to undergo training and start offering TFGT to breast cancer patients, and six members of the clinical genetics team who counsel patients from the ovarian and breast pathways. Interviewees expressed a range of opinions. Some, primarily oncologists, championed the introduction of TFGT in their clinic, while others, primarily breast surgeons, saw the introduction of TFGT as a threat to their professional identity and as negatively impacting their workload. Support for mainstreaming was related to the extent to which interviewees regarded this genetic information as informing personal treatment plans. We highlight some of the difficulties that may be encountered when introducing genetics into mainstream specialities and emphasise the need for further education of non-genetics specialists. Breast Cancer Now Grant no: [2016MayPR700]

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P19.05A

Managing uncertainty in cardiac genetics - An international survey

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Introduction: Multi-gene panel testing is useful in genetically heterogeneous conditions, including inherited cardiac pathologies. Extended panels increase diagnostic yield, identifying variants where pathogenicity is certain (ACMG (1) class 5), probable (class 4) and uncertain (class 3). Concerns exist regarding management of class 3 and 4 variants, particularly for conditions of oligo-genic inheritance or variable expressivity.

Methods: We developed an electronic questionnaire (www.surveymonkey.com/r/cardiacvariants) based on inter-disciplinary concerns to gauge international practice. This link was distributed to colleagues by email or via professional bodies.

Results: 126 respondents (21 countries) completed the survey, the majority from UK, US or Australia. 91(72%) were clinical genetics professionals, 12(10%) cardiologists and 15(12%) clinical scientists. Table 1 outlines circumstances where predictive testing would be offered to relatives of the proband. Considering class 4 variants, 101 (80%) counselled about possible future variant reclassification. With negative predictive tests, 88(70%) counselled about outstanding risks of developing the familial phenotype. 33(26%) would recommend discontinuing cardiology follow-up, while for 44 (35%) management decisions would depend on phenotype and family history. Most (67, 53%) respondents thought variant reclassification should be performed at least annually.

Conclusions: Considerable variability in management of families with class 3 and 4 variants exists. Decision-making relies on the phenotype, family history and genotype. Close multi-disciplinary working between cardiology and genetics is critical to accurately define phenotype and genotype.

1. Richards et al, Genetics in medicine. 2015;17(5):405-424.

Predictive testing

Predictive Testing	Yes	No	Affected relatives only
Class 5	122(97%)	2(2%)	1(1%)
Class 4	99(79%)	2(2%)	23(18%)
Class 3	12(10%)	30(24%)	82(65%)

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P19.06B

Patients at high risk of bowel cancer like the opportunity to receive, store and share confidential documents via a secure website - www.familyweb.org.uk

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Many relatives who share a high lifetime risk of cancer remain unaware of their opportunities for genetic testing or screening. Providing letters and verbal recommendations to patients are not necessarily sufficient to support effective

communication in families and methods for disseminating information to relatives are still under debate. The Family Web study investigated sharing digital documents securely online. Data from a cross-sectional survey (n=286) and telephone interviews (n=14) informed website content. Video recorded Think-Aloud interviews (n=12) elicited users' reactions to a website through three iterative phases. Patients at high risk of bowel cancer who had been advised to have regular colonoscopy and were aware that their diagnosis had implications for their relatives were interviewed while testing the website, commenting on its appeal and function. Recruitment was via NHS clinics or charity websites in the UK. Participants welcomed the opportunity to store and share personal information on the website but desired more support from health professionals, reporting the profound effect of the diagnosis on them and their family relationships. They wanted more information on a variety of topics to support themselves and inform their relatives. Key issues were: advice on a healthy lifestyle, talking to children, gene specific risks and accessing adequate cancer surveillance. The results indicate that use of a personalised web-based resource is acceptable to patients and meets a range of their information needs. This is important in the context of growing numbers of patients diagnosed with Lynch syndrome through tumour screening. Website funded by charity: Bowel Cancer West.

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P19.07C
Developing a competency framework to consent patients for genomic testing

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Introduction: Genomic medicine involves unique considerations with regards to informed consent, which have been highlighted in the 100,000 Genomes Project. Specific challenges include addressing the needs of different family members, data sharing protocols, and returning results including additional findings. It is important to promptly address the training needs of healthcare professionals that may be involved in consenting patients for genomic testing as it becomes increasingly part of standard clinical care.

Materials and Methods: Standardized consent training materials were developed by the authors and included Good Clinical Practice training as well as courses developed by Health Education England's Genomics Education Programme. The competency framework involved a three hour training session focussing on considerations for consent in the 100,000 Genomes Project, followed by the opportunity to observe and be observed in consent appointments as well as ongoing mentorship.

Results: 45 NHS staff members across the West of England region were trained, including genetic counsellors, nurses and specialist registrars. Overall, staff members felt that the framework provided appropriate information and guidance; however, some expressed concerns regarding the extent of their scientific knowledge and ability to address the wider familial implications of genomic testing.

Conclusions: Our experience delivering training across a region for a specific project highlights the skill gaps and knowledge required for healthcare professionals to address issues specific to informed consent for genomic testing. This emphasizes the need to embed a competency framework in order to enhance the provision of genomic medicine for effective patient and family-centred care.

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P19.08D
Creation of Ukrainian guideline of cystic fibrosis neonatal screening

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Introduction: The first national pilot project of cystic fibrosis newborn screening (CF NBS) was performed in 2012-2014 years. National program CF NBS is going restart in 2018. The aim of the study was to analyze preliminary results and approved strategy of next program for all specialists from 12 regional genetic centers.

Materials and Methods: IRT level results of 894152 newborns tested in pilot program was analyzed together with all diagnostic procedure of CF NBS.

Results: IRT retest investigated for 6943 newborns in pilot program. 95 patients with positive second IRT retest had confirmed CF diagnosis. DNA test was done for 75 patients. CF mutations were found in 92.59% of diagnosed patients. These results were discussed at national workshop with specialists from all 25 Ukrainian regions. There were approved basic principles of CF NBS within this framework for Ukraine: IRT/IRT/DNA.

Conclusions: Results analysis showed the advisability IRT/IRT/DNA scheme to screen CF in newborns in

Ukraine. Workshop results will be included in the national guideline with procedure of internal and external quality control systems. The increased CFTR mutations spectrum will be tested at the next CF NBS program. DNA test will be held in two state accredited centers. The development of a national guideline and the workshop is an important link in the education of specialists and improvement of their work quality.

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P19.09A

Adopting a gene through Human Disease Genes website series facilitates a clinical diagnosis for rare genetic disorders

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Human Disease Genes website series (HDG) is an international library of websites for professional information on the clinical consequences of novel variants in the human genome (http://humandiseasegenes.nl/). HDG is an initiative of the Human Genetics Department, Nijmegen, in collaboration with the University of Washington and the University of Adelaide. Each website is moderated by a dedicated team of clinicians and molecular biologists, and collects and provides up-to-date mostly unpublished clinical information about one specific gene or copy number variant. HDG aims to fill the gap between first publication of several cases and consecutive publication of a large review paper. Professionals can use the information of the new and rare disorder for counseling and will have the opportunity to share clinical data. Patients, parents and care-givers will find useful information on the disease and have the opportunity to share detailed clinical information through the website of our partner GenIDA. HDG is also a platform where researchers can collect and share (functional)data. Recently a new version of the websites has been launched which not only allows collection of phenotype data using HPO (http://humandiseasegenes.nl/wac/professionals/upload-clinical-information/) but also provides an up-to-date overview of the clinical data that have been collected (http://humandiseasegenes.nl/wac/graph-and-chart/). These tools will be highly valuable for the clinical practice. So far, over 60 genes have been adopted (http://humandiseasegenes.nl/gene-websites/) by more than 60 moderators world-wide (http://humandiseasegenes.nl/moderators/). If you wish to adopt a gene and join the team of moderators please, contact us at the poster or send us a mail to info@humandiseasegenes.com.

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P19.10B

Awareness of screening and diagnostic tests for Down syndrome in Bulgarian women at reproductive age

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Introduction: NIPT (non-invasive prenatal tests) are fast coming to the practice and aggressively offered by private companies. Nevertheless, the question remains - do women at reproductive age understand the possibilities and limitations of the different tests for Down syndrome and which are the key factors for preferring a certain test.

Materials and Methods: We present a survey among 300 randomly selected females from 18 to 47 years old, carried out both online and at the office for genetic counseling in the Laboratory of Medical genetics, Varna. Of all 59.3% are currently pregnant, 35.8% have been pregnant before, 4.9% not been pregnant.

Results: 73.2% of the women receive information from the obstetrician although high percentage are self-educated - 26.8%. In total 65.7% of the females think that this information is well or very well delivered. 66.1 % describe their understanding of biochemical screening for Down syndrome as good or very good compared to 41.6% who do not know what NIPT is. They are better informed about the amniocentesis - 56.2%, though only 13.7% would definitely undergo the procedure. The main argument when choosing the method for diagnosing Down syndrome is the accuracy of the test - 65.1% and only 1.2% point the price. However, the majority (77.3%) are willing to pay no more than 150 euro.

Conclusions: The results from the survey highlight the lack of knowledge about the offered screening and diagnostic tests for Down syndrome, especially NIPT. Better education and counseling of women during their pregnancy consultations are recommended.

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P19.11C
ERN-ITHACA Year 1: Opportunities and Challenges

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The 2011 EU Directive on Patients' Rights in Cross-border Healthcare aims to facilitate access to healthcare resources from across the EU and thus increase patients' management and treatment options. In response to this, 24 thematic European Reference Networks (ERNs) for rare disorders were formed in March 2017.

The ERN for Congenital Malformations and Intellectual Disability (ERN-ITHACA) seeks to improve access to diagnosis, consistent high-quality care, educational and research resources by facilitating expert exchange, bringing TeleHealth into clinical practice where required, establishing rare patient registries, encouraging engagement in pan-European research activities and creating new educational resources for professionals and patient groups. Here, we demonstrate some of our key initiatives in delivering patient benefit as part of ERN-ITHACA.

In year 1 there have been tangible benefits: interaction with patient groups helped us formulate an all-encompassing strategy; the web-based Clinical Patient Management System (CPMS) has been piloted successfully; expertise on care pathways and training initiatives has been consolidated and ITHACA has participated in large-scale EU projects, such as SOLVE-RD and the European Joint Programme.

However, there have also been challenges: the networks are conservatively financed by the EU and can therefore be accommodated by Health Care Providers (HCPs) from countries with existing management frameworks; the CPMS still presents many technical challenges; inclusion of new HCPs will strain the ERNs. We have developed strategies to overcome these challenges and the first year represents the first step in implementing changes which will have a positive impact on the care of patients across Europe.

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P19.12D
Clinical implication of *FMR1* intermediate alleles in a Spanish population

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FMR1 premutation carriers (55-200 CGGs) are at risk of developing Fragile X-associated primary ovarian insufficiency (FXPOI) as well as a progressive neurodegenerative disorder called Fragile X-associated tremor/ataxia syndrome (FXTAS). *FMR1* premutation alleles are also associated with a variety of disorders, including psychiatric, developmental, and neurological problems. While the clinical implications of the *FMR1* premutation alleles are well established, there is major concern regarding smaller CGG expansions known as intermediate alleles (IA) or gray zone alleles (45-54 CGG). Although several studies have hypothesized that IA may be involved in the etiology of *FMR1* premutation associated phenotypes, this association still remains unclear. The aim of this study was to provide new data on the clinical implications of IA. We reviewed a total of 17,011 individuals: 1,142 with premature ovarian insufficiency, 478 with movement disorders, 14,006 with neurodevelopmental disorders and 1,385 controls. Similar IA frequencies were detected in all the cases and controls (cases 1.20% vs. controls 1.39%, $p = 0.427$). When comparing the allelic frequencies of IA ≥ 50CGGs, a greater, albeit not statistically significant, number of alleles were detected in all the cohorts of patients. However, statistical analysis did not detect significant differences between any group of patients and controls suggesting that IA do not confer increased risk for primary ovarian insufficiency,

movement disorders, or neurodevelopmental disorder phenotypes. This study was supported by ISCIII [PI17/01067], co-financed by FEDER "una manera de hacer Europa" and AGAUR 2017 SGR1134 from the Generalitat de Catalunya and by grants from MINECO [BIO2011-27069] [SAF2013-49108-R].

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P19.13A

Skills and competencies in clinical genetics for the European general practitioner

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Introduction: The training programs of the specialty of General Practitioner (GP) remain heterogeneous at a EU level. Henceforth, in some European countries lack the skills and competencies in clinical genetics for the GP. This poster is a proposal of these skills and competencies.

Material and Methods: This study has been carried out through a qualitative group of twelve experts selected by these three criteria: GP specialists, interested in clinical genetics and from different Spanish regions. During a four-hour session was discussed what kind of skills and competencies should be included in the curriculum of the trainee for GP.

Results: As a result of the work carried out by a group of experts, certain skills and competencies have being identified for the curriculum of the European GP.

Conclusions: It is very important to adapt the current regulation across Europe in order to include specific skills and competencies in clinical genetic for the curriculum of the trainee of GP. A rotation of this professional during a month in a service of clinical genetics complementing the current competence-knowledge matrix would grant better tools to improve the quality of the service provided to patients.

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P19.14B

Translating the German Commission on Genetic Testing (GEKO) guideline for the requirements of the qualifications and the contents of genetic counseling into OB/GYN specialist practice - Results from the GenBIn2 project

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Background: The German Act on Genetic Testing stipulates the requirements of good practice with regard to safety, informed consent and free decision-making for people undergoing genetic testing. The act required the GEKO to issue a guideline on requirements for competence in genetic counseling within the scope of each medical subspecialty (2011). No empirical data are currently available on how the GEKO guideline is applied at point of care.

Objectives: To provide preliminary data for assessing the translation of the guideline.

Methods: (i) key-areas for an assessment were identified by a multidisciplinary expert group; (ii) development of a questionnaire based upon key-areas (iii) a pre-study/inquiry guided by the questionnaire.

Results: 150 OB/GYN representing different types of practice participated in the inquiry. 80% had obtained the qualification for genetic counseling within their specialty. 48% reported changes in the management of patients, 61% reported more referrals to genetic specialists for genetic counseling. Genetic counseling provided by OB/GYN mostly include: counseling before First Trimester Screening (ETS) (76%) and after ETS with normal results (77%), before prenatal ultrasound for fetal anomalies (64%), before NIPT for Trisomy 21 and after NIPT with normal results (57%). Additional work load, time constraints in busy practice settings and the guideline conflicting with established approaches to delivering care were reported. Positive responses include: increased sensitivity for skills required for genetic counseling and increased referrals to genetic specialists.

Conclusions: By identifying numerous challenges the inquiry helps to better understand factors influencing the translation process and provides groundwork for future assessment studies.

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P19.15C

Factors affecting compliance to genetic counseling in the Israeli Bedouin population

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Background: Genetic counseling is mostly carried out in the framework of hospitals. With recent massive progress in genetic technologies and propensity in genetic testing, there is growing need to make the genetic services more accessible in community medicine in order to increase responsiveness. This is of specific interest in inbred rural communities, where consanguinity leads to high incidence of hereditary diseases, yet mobility to central medical centers is cumbersome.

Objective: To assess in the rural Bedouin Israeli community the compliance of arrival to genetic counseling in a central medical center following nurse-mediated early interviews and referral.

Methods: Prospective study. Data collected from records of couples visiting genetics-specialized nurses at public community clinics, including personal interviews, clinical, demographic and obstetric data, and family history of genetic disease variables.

Results: Of the 1754 women reaching initial interview by the genetics-trained nurse, 684 (40%) needed further professional genetic counseling at Soroka medical center. Reasons for further formal genetic counseling were: Medical problems detected during current pregnancy; Consanguinity; Family history of genetic diseases; previously known carrier status of couple. Of the 684 women referred to formal genetic counseling, only 360(52.6%) arrived. Reasons for failure to arrive were assessed.

Conclusion: In a population with high incidence of intermarriage and genetic diseases, it is recommended to integrate a genetic counseling services in the community health system to increase responsiveness to genetic counseling and testing in a timely manner, adapted to religion and culture. Dedicated teams should be trained and continuous activity promoting public awareness is essential.

R.O.I. abo rabia: None.

P19.16D

An alternative pathway for countries without a training program for genetic counsellors: the Swedish experience

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In Sweden there is currently no academic education at master level in genetic counselling, which is recommended by the European organizations promoting professional regulation. As a result, professionals with different backgrounds and experiences are employed as genetic counsellors in Sweden. There is a need to harmonize the quality and standards for practice.

The Swedish Society for Genetic Counsellors, SFGV together with the Swedish Association for Medical Genetics, SFMG have determined standards, and created a career development pathway for genetic counsellors.

The result is an individual training program leading up to certification for genetic counsellors in clinical practice in Sweden. Each professional has an educational plan, and there are three different levels of employment within the genetic counseling profession. The first level is an employment according to the individual´s basic profession, the second level is an assistant genetic counsellor and the third level, as a genetic counsellor, requires an academic education equivalent to one year at an advanced level in the nursing or biomedical field, and approval as a certified genetic counsellor according to the SFMG-standards.

Conclusion: Professional certification is a way of assuring that an individual has the mandatory knowledge, skills and abilities to provide quality of patient care and safe practice. We describe an alternative pathway for national regulation of genetic counsellor career development and certification process that may support other countries in providing national regulation of genetic counsellors where the profession is still emerging, and where no educational route.

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P19.17A

Complementariness between medical geneticists and genetic counsellors: its added value in genetics services in Europe

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Clinical genetic services have progressed significantly the last few decades. This has led to the need for non-medical healthcare professionals working as genetic counsellors in Europe and worldwide. However, there is no unified approach to genetic counsellors' role in healthcare services in Europe, as in most countries the profession is still emerging and the educational backgrounds diverge noticeably, within and between countries. This qualitative study aims to describe the potential added value of genetic counsellors in clinical genetics teams and to explore their tasks and responsibilities in different European countries. A total of 143 participants providing genetic counselling in Europe at the time of the survey responded. The results show differences in activities of genetic counsellors, although there is a wide range of roles which are similar. The ability to establish a quality relationship with consultands was frequently mentioned as one of the strengths of genetic counsellors, as well as a patient centred approach. It is believed that genetic counsellors add a more holistic approach of psychosocial and familial dimensions of genetic concerns to the multidisciplinary teams. This study provides examples of successful integration of genetic counsellors in teams, as complementariness with medical geneticist became clear in several cases. Although the added value of genetic counsellors was manifested, professional recognition of genetic counsellors across Europe is still needed in order to support the quality of patients care and safety of practice.

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P19.19C

Genetic professional skills performance in nursing: A preliminary study in highly educated nurses

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Aim: To study factors associated with genetic skills performance in nursing in highly educated nurses in Israel.

Methods: Data were collected during a conference for professional associations in nursing in Israel. The participants were asked to complete a set of anonymous questionnaires which included, genetic knowledge, mathematics literacy, sense of genetic epistemology, openness to technological innovations, attitude towards the importance of assimilating genetics skills in nursing, and the extent they perform these skills in the routine nursing work.

Results: Participants included 81 nurses aged 46.5±10.6 years on average, most of them were Israeli born, Jewish, secular, working in hospitals, and ~70% holding a Master's degree. The mean genetic knowledge was low (53.4% ±13.43), as was the mathematics literacy (61.3%±25.3). Nurses reported of low assimilation of genetic skills in their routine work (1.9±0.78; range, 1-4), although their overall attitudes towards the importance of genetics in nursing were positive. Positive correlations were found between a sense of epistemology about genetics, and attitudes towards the importance of genetics in nursing, with assimilation of genetic skills in routine nursing work. These variables added 33.4% to the explained variance in the hierarchical regression model for variables predicting genetic skills performance in nursing. Additionally, mathematical literacy correlated with genetic knowledge, and older nurses held more positive attitudes towards the importance of assimilating genetics skills in nursing.

Conclusion: Although the nurses in this study were highly educated, we found disappointingly low scores in genetic knowledge and in performance of genetic skills in the nursing routine work.

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P19.20D

The evolving role of the clinical geneticist facing new technologies: the opinion of patients from the AnDDI-Rares network

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The arrival of new diagnostic technologies and genomic medicine lead to important changes in genetic clinics. An expected growing number of indications of tests raise the question of the prescription of pangenomic tests by non-geneticists. In that case, clinical geneticists are worried of the possible reduced quality of patient information, in particular the limited time dedicated to the implications of test results, genetic counselling, but also new issues including secondary data and management of VUS.

In this context, a survey was done to study the preferences of patients for the circuit of prescription and transmission of results of NGS tests in a clinical diagnostic setting. The survey was sent to a group of 50 patients organizations of the AnDDI-Rares network (developmental disorders and intellectual disability). 404 questionnaires were collected. More than 80 % of the respondents had met a clinical geneticist in their diagnostic odyssey. 93% of them agreed that geneticists should have a primary role in the prescription of pangenomic tests, in particular because genetic teams offer multidisciplinary cares (genetic counsellors, psychologists, social worker). However, 72%, were also in favour of NGS tests being prescribed by a non-geneticist medical specialist, in particular because he knows better his patient, and have shorter delay for accessing to consultations. In these cases, 70% of the respondents would like a consultation with a geneticist for transmission of the results. With NGS development, the whole patient care pathway needs to be redesigned and professional training reconsidered to face this the arrival of genomic medicine.

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P19.21A

"Experience is the teacher of all things"- upskilling the genomics workforce in variant interpretation

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Introduction: Rapid advances in genomics are bringing benefits to healthcare but not without challenge. Key among these is the need for a workforce that can deliver the benefits of genomic medicine to patients.

Methods: In the demonstration phase of Melbourne Genomics, an alliance of 10 leading healthcare and research organisations, an immediate need, and opportunity, was identified to develop a workforce to guide genomic sequencing into healthcare across Victoria. To address this we have developed a multi-faceted workforce development program providing opportunities for learning experiences that enhance and augment clinical and laboratory practice, focussing on engagement with real-life problems relevant to practice or skills-based training.

These include:

- Provision and evaluation of genomic sequencing to patients through defined flagships providing a multi-disciplinary environment for experiential learning;
- A 12 week cross-training program for medical scientists in variant curation and reporting;
- Research placements in genomics for advanced trainee clinical geneticists;
- Research, clinical and laboratory placements for non-genetic medical specialists that address an authentic problem drawn from their practice.

These experiential placements are being augmented with

- Interactive workshops in germline and somatic variant curation
- Development of on-line modules in variant curation and interpretation

Results and Conclusions: Ongoing qualitative evaluation of the program reveals consistently high levels of engagement and learning. Quantitative evaluation of structured workshops measured improved knowledge and skills for all participants with over 90% reporting high levels of satisfaction. This success demonstrates the effectiveness of interactive, participant-centred approaches in developing a sustainable genomics workforce development strategy.

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P19.22B

Custom-designed free educational Clinical Genomics apps for smartphones & tablets: their international uptake and further development

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Educational and self-assessment smartphone apps that explain (and test knowledge of) commonly used genomics and bioinformatics terms and acronyms, were created by the authors. These non-profit Clinical Genomics guide and quiz apps have been made **available, completely free of charge, via the official Apple and Android app stores** (search for: "Clinical Genomics"). They provide immediate clear explanations of **genomics-related terminology (such as "FASTQ", "BAM", "GVCF", "BED files", "GATK" and "BWA")** that are otherwise not easily accessible.

Creating these multi-platform apps was time-consuming. Highly positive feedback has, however, been received from physicians, genetic counsellors, laboratory scientists and students, internationally. **The apps are used by postgraduate students** to assist their understanding eg during lectures or when reading scientific papers. For instance, they are used to assist understanding of key Clinical Genomics concepts and terminology by students on the internationally popular Medical Genetics & Genomics Masters programmes at Glasgow University.

The apps are also used by professionals, who increasingly receive complex genetic laboratory reports that must be interpreted for patient benefit.

The apps have been **approved by the ESHG, the Scottish Genetics Education Network (ScotGEN) and by DSDnet**. They have been downloaded thousands of times, across approximately 50 countries and have recently been shortlisted for a national higher education "Innovation Technology Excellence" award. Further programming and development of the apps is currently underway, to include explanations of additional terms and concepts. The authors would be grateful for further feedback and for suggestions regarding any more terms that could be incorporated. **Please email: edward.tobias@glasgow.ac.uk**.

E.S. Tobias: None. **A.P. Tobias:** None.

P19.23C

Genomics in mainstream clinical pathways

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The 100,000 Genomes Project in the UK has established and refined many of the processes around the delivery of whole genome sequencing at scale. The next objective is the implementation of these technologies as an integral part of routine healthcare in specialities outside genetics thus promoting equitable access to these technologies. This requires 'mainstream' clinicians to adopt some tasks which are currently the province of clinical geneticists. In March 2017, we held a workshop bringing together clinical and genetics specialists and specialists from other clinical areas including cardiology, ophthalmology, paediatrics, respiratory and renal medicine, to evaluate the particular issues that arise at two interfaces: patient identification and referral for testing; and interpretation of results and clinical decision making. We found that most clinicians will need a lot of support to identify patients who may benefit from testing and decide which test to request. The capture of necessary clinical and phenotypic information at the point of referral is problematic. Examples of supporting methods were shared. Similarly, barriers to involving mainstream clinicians in interpretation include practicalities (such as geography, timing and length of consultation) and lack of required expertise, which may be outside their clinical area. Support for clinical decision making based on genomic test results would be vital for effective implementation. For equitable implementation of genomic testing across the health system detailed attention must be paid to developing new clinical pathways in each speciality and the necessary support systems. Our report can be downloaded from http://www.phgfoundation.org/report/genomics-mainstream-clinical-pathways.

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P19.24D

Genetic counselling in hereditary diffuse gastric cancer: economical and psycho-social impact

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Introduction: Hereditary Diffuse Gastric Cancer (HDGC) syndrome is caused by *CDH1*-germline mutations and carriers have high-risk to develop early-onset diffuse gastric cancer (DGC) in both genders and lobular breast cancer (LBC) in females. HDGC is deadly for those expressing clinical phenotype, but presymptomatic testing allows disease prevention or early-diagnosis in mutation-carriers through risk-reduction gastrectomy and yearly breast MRI, discharging ~50% of non-carriers. As Health-Care-Provider of ERN-GENTURIS, we aim to evaluate the economic impact of genetic counselling, presymptomatic diagnosis and multidisciplinary care for the National Health Service (NHS), and the clinical and psycho-social implications for HDGC families.

Material and Methods: We evaluated outputs of structured oncogenetics and high-risk consultations in 111 individuals from 7 HDGC families by consulting clinical/financial records, and assessed its psycho-social impact by applying an emotional-distress-scale (HADS) to 70/111 individuals.

Results: From 2011-2016, we screened 111 individuals, from which 53% tested negative (n=58;30M:28F) and were discharged from follow-up each costing 200€ to the Hospital. 47% (n=53;26M:27F) tested positive. From 7 probands, 2 were diagnosed with LBC and remain alive after curative surgery, while 5 diagnosed with DGC are deceased. The hospital expenses with probands range from 30-50K€ independently of cancer type. Costs with carriers opting for prophylactic approaches range from 4-8K€ (higher cost/females), except if disease is identified after the first high-risk consultation, raising the cost to 27K€ but life-saving. Those opting for surveillance, cost ~1K €/patient. Concerning psychologic impact, carriers demonstrate higher levels of distress than non-carriers.

Conclusion: Structured healthcare in HDGC economically benefits NHS, is life-saving for carriers, reassuring non-carriers.

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P19.25A

Comparison between the attitudes of genetics professionals and patients towards incidental findings from whole-genome or whole-exome sequencing

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There is a consensus that whole-genome sequencing (WGS) and whole-exome sequencing (WES) will improve in accuracy and decline in price, eventually becoming an integral part of clinical medicine. In clinical genome sequencing, there is a potential for the recognition of incidental findings that are unrelated to the indication for ordering the sequencing but may nonetheless be of medical value. Here, we aimed to compare the attitudes of genetics professionals and patients towards incidental findings from WGS/WES, including a hypothetical situation in which a patient/family declines to receive all the incidental findings. A web-based survey was conducted with 20 genetics professionals (medical geneticists and genetic counselors) and 30 patients from King Faisal Specialist Hospital and Research Center in Riyadh, Saudi Arabia. Data collection took place between the 2nd of April and the 4th of May 2017. The results demonstrated that 55% of genetics professionals would not share all WES/WGS results with patients and they preferred to focus on actionable results that yield benefits such as medical treatment and disease prevention. However, 73.3% of patients were interested in receiving all the raw genomic data for themselves and their children. References 1- Alkuraya, F. S. (2014). Genetics and genomic medicine in Saudi Arabia. Molecular Genetics and Genomic Medicine. 2- Kalia et al., 2017. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update a policy statement of the American College of Medical Genetics and Genomics.

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P19.26B

Informing relatives at risk of inherited cardiac conditions: attitudes of healthcare professionals, probands and relatives

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Inherited cardiac conditions (ICCs) can lead to sudden cardiac death (SCD) at young age. Cardiac monitoring and/or predictive genetic testing is advised to at-risk relatives to prevent SCD. Usually, probands are asked to inform their relatives with a family letter. This study investigated attitudes towards this family-mediated approach in ICCs to use for potential improvements. A qualitative study design was used. Two online focus groups with 27 healthcare professionals (HCPs), including cardiologists, clinical geneticists, genetic counsellors and psychosocial workers, and 25 face-to-face semi-structured interviews with probands and relatives were independently analysed by two researchers using a thematic approach. Main findings show that it is generally preferred that probands inform their relatives. However, both HCPs and probands struggle with the dependency and psychological and practical burden on probands to inform their relatives. HCPs think a more active role of HCPs in informing at-risk relatives is needed to overcome these barriers. Probands and relatives ideally prefer a tailored information provision strategy to inform at-risk relatives, adjusted to family dynamics and personality characteristics. In contrast, HCPs prefer uniformity in procedures to restrict the workload. To summarize, although in most cases it is preferred that probands inform relatives, several barriers are perceived. Based on these findings, we are conducting a randomized clinical trial to evaluate the uptake and acceptance of a tailored approach, compared to the family-mediated approach. In this tailored approach probands can choose which relatives they will inform themselves and which relatives they prefer to be informed by the counsellor.

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P19.27C

A qualitative study exploring patient attitudes towards novel therapies for inherited retinal dystrophies

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Introduction: There is currently no treatment or cure for inherited retinal dystrophies, yet numerous clinical trials are underway investigating novel therapeutic strategies. This study aimed to explore the awareness and opinions of new therapies among IRD patients in South Wales.

Materials and Methods: Ten participants with diagnoses of IRD were recruited through the Genetic Eye Clinic at Cardiff and Vale University Health Board (C&V UHB). Semi-structured qualitative interviews explored patients' attitudes towards novel therapies for IRD. Interviews were transcribed verbatim and analysed using thematic analysis.

Results: Interviewees' attitudes were classified into three broad themes: hope, despondency, and 'knowledge is power'. Whilst hope was expressed universally among participants, this was also accompanied by concerns regarding the risk involved in therapy uptake and the lengthy timescale of development. There was also an appetite identified for access to more information regarding therapy development from trustworthy, accessible sources. Although participants varied in the extent to which they felt anticipation versus resignation it was universally acknowledged that all would draw empowerment from being provided with up-to-date, accurate information.

Conclusions: This study suggests there is a need for improved dissemination of accessible information on novel IRD therapies through face-to-face as well as online platforms. The development and validation of an ophthalmic genetic-specific PROM may help to ensure the broad and complex needs of IRD patients are met. A better-informed IRD patient population could improve supply to clinical trials and ultimately, aid in the successful development and clinical implementation of novel IRD therapies.

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P19.29A

An interactive application to support practical teaching in medical genomics and bioinformatics

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Medical students usually find molecular genetics, genomics and bioinformatics difficult and remote from their future clinical plans. However, their orientation in these topics is necessary as genetic testing is rapidly penetrating into all medical specialties. Patient involvement and practical laboratory work can enhance teaching, but obstacles limit their broader implementation. Computer simulations can instead be used to ameliorate the teaching process.

We created an electronic teaching application simulating the process of molecular genetic diagnosis of a monogenic disorder in a family. Thirteen tasks presented on a sequence of web pages deal with the clinical assessment of pedigrees and phenotypes, clinical diagnosis, design of a laboratory test, identification of a mutation using capillary sequencing,

assessment of pathogenicity and clinical relevance of the mutation, and final confirmation of the diagnosis. The tasks employ publicly available bioinformatic resources used in the daily routine of medical genetics departments worldwide.

Feedback from students was obtained using a test administered before and after the practical. Most comments on the practical were very positive. The answers to specific test questions were classified as very good, satisfactory, or unsatisfactory. There was a significant improvement after the practical (on average by one grade) of knowledge and skills of the students in medical molecular genetics, genomics and bioinformatics.

The feedback is used to improve potentially problematic sections of the practical. A pilot version of another teaching application, illustrating the workflow of whole-genome analyses, is under development.

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P19.30B

Development of a hands-on genomics educational tool using mitochondrial DNA PCR-free enrichment and MinION-based species apportionment

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Introduction: Because of the low cost, laptop operability, and the USB-powered compact design of MinION (Oxford Nanopore Technologies), using cutting-edge Next-Generation Sequencing (NGS) technology in field experiments and the classroom is a reality. By leveraging the benefits of using mitochondrial DNA (mtDNA), here we devised the protocols and materials for a NGS hands-on training with educational purposes.

Materials and Methods: We assessed two procedures for facilitated mtDNA enrichment from foods: QIAprep Spin Miniprep Kit (QIAGEN) and the classic alkaline lysis protocol for plasmid isolation. Enrichment of mtDNA *vs.* nuclear DNA was evaluated by quantitative Real-Time PCR (CFX96 Touch, BioRad) using primers from eukaryotic conserved regions. PCR-free libraries were constructed with Rapid Sequencing Kit and sequenced with MinION. A Jupyter notebook-based pipeline with plain contextual explanations was designed to seamlessly progress from event-level data and obtain species abundance.

Results and Conclusions: Overall, protocols performed similarly enriching mtDNA directly from DNA extractions, both from animal and plant species. We anticipate that the choice of enrichment will depend on the attendees' educational level and the available laboratory equipment. Sequencing of a mock specimen (artificial mixture of foods) after a simple 10-minute library preparation allowed identifying all species with minimal deviations from the expected. We envisage this hand-on experience as a learning-by-doing instrument for transdisciplinary education adapted to basic laboratory resource settings.

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P19.31C

Tricky situation and diagnostic odyssey solved by combination of "make a point" genetic counseling and NGS

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Encephalopathy due to defective mitochondrial and peroxisomal fission-1 (EMPF1) is characterized by delayed psychomotor development and hypotonia that may lead to death in childhood. Many patients develop refractory seizures, consistent with an epileptic encephalopathy, and thereafter show neurologic decline. The age at onset, features, and severity are variable. We report on, 40 years old woman pass through our Genetic Counseling, due to positive anamnestic data regarding presence of intellectual disability, transient ischemical episodes and myoclonical seisures in sibs. These signs and simptoms were present in first son (exitus at 3 years), as also in second son (exitus at

11 years; the DNA samples available). The children were generated by different fathers. A hypothesis of EMPF1 was advanced. The sequencing of *DNM1L* gene has been performed on the proband's, mother's and DNA of 2nd father. The known patogenetic mutation c.1085G>A (p.Gly362Asp) and VUS c.1535T>C (p.Ile512Thr) in the *DNM1L* gene were revailed in proband's DNA. The sequencing of mother's DNA resulted in presence of only VUS c.1535T>C (p.Ile512Thr), while in father's DNA both variants were absent. On the basis of these observation it was fairly difficult to interpret this situation as *de novo* autosomal dominant variant. The further analysis by NGS has been performed, heterozygosity of c.1085G>A (p.Gly362Asp) variant was revealed in 5% of mother's DNA. All variants have been confirmed by Sanger sequencing. Herein and in n Post-test Genetic Counseling the results and in depth explanation of germinal mosaicism have been discussed.

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P19.33A

f-tree: A family health history tool available from the Iwate Tohoku Medical Megabank Organization

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Introduction: The Tohoku Medical Megabank project aims to create a next-generation personalized healthcare system. We designed a questionnaire-based pedigree-drawing software program named "f-tree" to enable users to accurately and rapidly collect genealogical information and assemble pedigrees.

Materials and Methods: f-tree is a stand-alone application written in ActionScript 3.0. Because it was built as a cross-platform runtime system using Adobe AIR, it is important that Adobe AIR be installed prior to the installation and use of f-tree.

Results: f-tree is a bilingual software designed in Japanese and English, and complies with international nomenclature guidelines for human pedigrees. It can be used to assemble pedigrees in the following manner: 1) Upon system startup, the user is prompted to provide information regarding the presence or absence of children. f-tree can assemble a pedigree that comprises data for up to five generations, and places a couple with and without children within the third and fourth generation of a given pedigree, respectively. 2) Next, an interviewer may assist the user to complete a multiple-choice questionnaire to collect genealogically pertinent information for the user and their relatives over five generations. 3) f-tree then automatically creates a pedigree chart, which can be reviewed on the screen in real time.

Conclusions: The f-tree software program has been made available for free on the Iwate Tohoku Medical Megabank Organization website (http://iwate-megabank.org/en/genetic/). Until date, this program has been downloaded more than 3,000 times since its release on March 19, 2015.

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P19.34B

Comparison between different screening strategies for the detection of all fetal karyotype abnormalities

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Introduction: For T21,T18,T13 Cell-free DNA (cfDNA) tests have a higher detection rate (DR) and a much lower false positive rate (FPR) than traditional serum±ultrasound screening (TSS). However, TSS can pick up unexpected additional 'off-target' chromosome abnormalities consequent to the higher reflex invasive testing rate. The aim of this study is to compare the detection rates of all (target and off-target) fetal karyotype abnormalities at birth by cfDNA and TSS.

Materials and Methods: Using a model derived from our laboratory data, we predicted the DR of any karyotype anomalies at birth in pregnancies with no risk factors other than maternal age (MA) for seven screening strategies, including different TSSs, cfDNA tests (±SCAs) and contingent/sequential approaches.

Results: CfDNA testing for T21,18,13+SCA (cfDNA-TXY) has the highest DR for all karyotype abnormalities at all MAs. In young women, cfDNA for T21,18,13only (cfDNA-T) has a lower DR than any TSS due to the 5%FPR

resulting in the identification of a greater proportion of off-target abnormalities; cfDNA-T equals TSSs at a MA of 38y, when trisomies dominate the risk.

Conclusions: Distribution of the chromosome abnormalities is different at different MA. Therefore, although two strategies may show approximately the same overall DR, TSSs favour the detection of a broad array of 'off-target' abnormalities, at the cost of a consistent individual residual risk, while universal cfDNA tests efficiently detect common aneuploidies with a limited view of 'off-target' abnormalities. These results are useful for the development of screening implementation economical models for public health policy decision-makers.

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P19.35C
Factors influencing the decision whether or not to have prenatal genetic testing in pregnant women having children with Down syndrome

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In Japan, non-invasive prenatal testing (NIPT) has been available as a clinical research since the introduction in 2013. Shinshu University Hospital does not participate in this clinical research, and offers careful prenatal genetic counseling (GC) for the decision whether or not to have amniocentesis-based fetal karyotyping by certified genetic counselors (CGC) and clinical geneticists specializing in chromosomal or genetic disorders. In this study, we report our experiences about prenatal GC to the clients having a child with Down syndrome (DS). From medical records of Center for Medical Genetics, Shinshu University Hospital from April 2013 to December 2017, we identified 8 pregnant women who had a child with DS and received prenatal GC. The average age of the cohort was 36.3 years (range, 32−41). Four of them (50%) had prenatal genetic testing and the others declined the testing. We reviewed factors influencing the decision whether or not to have the testing from their narratives. Reasons for choosing the testing were described as "cannot raise one more child with disabilities", and "request for the testing by the child's grandparents". Reasons for not choosing testing were described as "don't like to terminate the fetus", and "can make a happy family regardless of disabilities of the child". In the setting of prenatal GC for the clients who had children with DS, we have to aware that they could have ambivalent feelings about the burden and the value of raising children with DS.

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P19.37A
Project machine: a unique approach for industrial medical education

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Introduction: 'Project Machine' is a targeted project of our Medical Genetics department. It's established from undergraduate medical students in our university. The aim was to provide them a systematic way of conducting pilot projects to intend innovation and patent targeting academic improvement by teaching them on basic and advanced laboratory methods in Molecular Genetics.

Materials and Methods: Project Machine consists of 8 students from 3 different medical classes (Phase 2,3,4). The Project Machine group received hands-on training in eleven different subject projects in two years. Trainings were given by Medical Geneticist, the first Author. All of these activities were done as a team work particularly during their free studying times besides active medical education. Students were evaluated by Medical Scores (before and after participating machine), Laboratory works (n=30) and academic skills (n=17) completed. The *t* test and *Chi* square tests were used for statistics.

Results: The average score of the students who participated in the project machine increased significantly (79,24±8,961;min-max:56-98)(p = 0,021). The average molecular techniques learned were 17,75±8,598 (min-max:6-29). The average academic skills they succeed were 8,25±5,523 (2-15) including 3 domestic and 1 international awards won and 11 projects has finished during project (min-max:9-31 months) with an accepted publication.

Discussion: Participation of medical students in scientific studies by mutual interaction with academicians during their

medical education might lead more successful, prompt, and enhanced skills to overcome hard working and time consuming jobs in an active team work system. Hence, our study might be pilot model for industrial medical and genetic education.

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P19.38B

Psychiatric Genetic Testing - An emerging, interdisciplinary field

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Psychiatric disorders such as schizophrenia and bipolar disorder are common and highly heritable. Despite this high heritability it has taken many years to begin to identify underlying genetic risk factors. However, in the last decade major advances in psychiatric genetics have been driven by both technological advances (e.g. cost effective array-based genome-wide genotyping and next-generation sequencing technologies) and the establishment of large-scale, international consortia. Of particular relevance has been the identification of copy number variants (CNVs), such as 22q11.2 deletions, that confer substantial risk for psychiatric disorders. Findings such as these have sparked an interest in implementing genetic testing for patients with these disorders in routine clinical care. Psychiatric Genetic Testing (PsyGT) is a newly emerging and interdisciplinary field. Across Europe experts with varying backgrounds (e.g. Genetics, Epidemiology, Psychiatry) have assembled to facilitate pan-European research relevant to PsyGT and to help pave the way for its clinical implementation. Our work will address: (i) establishing a list of genetic factors for which it is scientifically/clinically appropriate to offer genetic testing; (ii) defining patient inclusion criteria for e.g. CNV testing; (iii) discussing the potential challenges of PsyGT (e.g. informed consent; reporting back incidental findings to a patient with a psychiatric disorder); (iv) promoting research into the potential positive and negative effects of PsyGT for patients and their family members; (v) increasing the "psychiatric genomic literacy" among all relevant stakeholders as genetic counsellors and psychiatrists report that they feel ill-equipped to educate patients and their family members about the genetics of psychiatric disorders.

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P19.39C
Improving rare disease identification and coordinating health and social care priorities

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Introduction: Rare diseases, present in $<^{1}/_{2,000}$ persons with a genetic cause, affect ~5% of the population in Northern Ireland (NI). Following public and stakeholder consultations, a NI Rare Disease Implementation Plan was published stressing 51 key commitments to improve medical and societal challenges specifically associated with rare diseases.

Materials and Methods: Mixed methods were employed to seek views from a range of stakeholders to recognise good practice, identify where urgent change is required, and prioritise short/medium/longer term goals for rare diseases in NI. The establishment of our NI Genomic Medicine Centre (2015) and applying multi-omics approaches to improve the speed and accuracy of diagnosis for individuals with rare diseases has revealed important issues.

Results: Differences were noted depending on the method of data collection. Common themes consistently included a frequently tortuous path to diagnosis, difficulty finding details for health and social care contacts, and challenges accessing relevant information in suitable formats.

Conclusions: Current health and social care systems in NI are not designed to optimise care for rare diseases. The development of our digital integrated care record (http://www.hscboard.hscni.net/encompass/) is offering unprecedented opportunities to integrate effective and sustainable strategies to improve rare disease identification, optimise care and service provision, provide relevant information to individuals living and working with rare diseases, and establish effective multi-disciplinary communication networks.

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P19.40D
Testing the ORPHA-code based Beta-Masterfile using data from the Belgian Central Registry of Rare Diseases (CRRD) for the use case of rare diseases patient data sharing at EU level

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Since 2016, 5 Belgian genetic centres started to provide data to the CRRD for all patients with a genetic diagnosis (confirmed or provisional). ORPHA-codes (preferably) or codes cross-referenced to ORPHA-codes are used. One objective of the CRRD is data sharing at EU level for epidemiological and other purposes.

However, lack of standardised use of appropriate rare disease (RD) coding across the EU is hampering data sharing. To allow data sharing, RD-ACTION WP5* members propose the use of a table, retaining only ORPHA-codes at disorder level in the Orphanet classification of RD. The table is called "Beta-Masterfile", a tool serving RD-coding and exploitation purposes prepared by the above-mentioned group.

We analysed the diagnosis-related information from the current CRRD dataset and evaluated the future exploitability at EU-level using the above-mentioned table.

Non-rare cases (~4%) were excluded from further analysis. For only 11 of 1633 cases analysed, a conversion into ORPHA-codes was necessary. ~65% of RD diagnoses had a confirmed status.

Considering that subtype ORPHA-codes are convertible into their parenting ORPHA-code, data from ~80% of CRRD patients could be shared at EU level, albeit losing more detailed coding information for ~6% of them. For both provisional and confirmed diagnoses, the use of ORPHA-codes at group-of-disease level is about 20%. The relatively high use of codes at group-of-disease level for confirmed diagnoses should be further investigated in order to obtain better quality of coding.

*WP5: "Steering, maintaining and promoting the adoption of ORPHA-codes for Rare Diseases across member states"; EU's joint action '677024/RD-ACTION'

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P19.42B

Triage in a Clinical Genetics setting - investigating consistency within and between units

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Background: The triage process aims to ensure provision of appropriate care to patients, within an acceptable time-period, depending on urgency of referral. Clinical Genetics is unique regarding breadth of types of referrals, and age range of patients (pre-natal, paediatric or adults). Referrals are complex, and ensuring consistency of triage can be difficult.

Aim: To compare triage practices within and between clinical genetic centres in UK (n=4) and Republic of Ireland (n=1).

Methods: Thirteen simulated referrals were created (TMcV, SAL) based on common real-life referrals to Clinical Genetics services, and distributed to each centre for "triaging". Individuals were asked to pick from the following options: offer appointment (face-to-face/telephone); hold referral pending further information; manage referral by letter/telephone call; or reject referral. For patients offered appointments, participants were asked to specify urgency (priority/routine) and clinician (consultant/counsellor). Data regarding staffing levels and waiting times for each centre was noted.

Results: Inconsistencies were noted within and between units (table). In 9/13(69%) referrals, clinicians in Centre1 were the least likely to offer face-to-face appointments. Centre1 had the lowest number of consultants/head of population.

Conclusions: Clinical triage is a somewhat subjective process. Institutional factors, including staffing levels, local practices; and patient factors, including age of patient/parent (family planning considerations), indication for, and type of, referral (predictive/diagnostic), can modify triage decisions. Standardised guidelines for triage are required to ensure equitable and appropriate service provision.

Proportion of Clinicians offering Face-to-Face appointments

	Referral	Centre1	Centre2	Centre3	Centre4	Centre5
1	Copy Number Variant	6(67%)	9(100%)	5(100%)	10(100%)	6(100%)
2	Hypermobility	0(0%)	8(89%)	3(60%)	4(40%)	2(33%)
3	Intermediate FMR1 allele	7(78%)	7(78%)	5(100%)	10(100%)	6(100%)
4	Trisomy 21	0(0%)	9(100%)	6(100%)	9(90%)	5(83%)
5	Adult with Intellectual Disability	5(56%)	6(67%)	3(50%)	9(90%)	6(100%)
6	Predictive BRCA (familial variant information unavailable)	4(44%)	9(100%)	8(89%)	7(70%)	3(50%)
7	Cervical cancer	0(0%)	2(22%)	0(0%)	1(10%)	0(0%)
8	Isolated Cleft lip	0(0%)	2(29%)	1(14%)	7(70%)	3(50%)
9	Congenital Heart Disease (unspecified)	0(0%)	3(33%)	2(40%)	7(70%)	6(100%)
10	Pregnant woman, Family history of Duchenne MD	8(89%)	9(100%)	6(100%)	10(100%)	6(100%)
11	Hereditary Haemochromatosis	3(33%)	3(43%)	0(0%)	6(60%)	4(67%)
12	Mitochondrial disease (pre-symptomatic)	4(44%)	3(43%)	2(40%)	9(90%)	6(100%)
13	Neural Tube Defect	3(33%)	7(88%)	4(57%)	4(40%)	4(67%)

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P19.43C

Young adults' perceptions of the implications of their hereditary visual impairment: A Cape Town based study

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Introduction: Globally there are 39 million blind individuals and 246 million with low vision. A third of genetic conditions involve the eye. Knowing that one's condition is genetic presents challenges for individuals and leads to a sense of obligation regarding life plans. As young adults with hereditary visual impairment must make choices regarding relationships, having children, further education or occupation and independence, which sets the foundation for adulthood, it is important to focus on them. The study explored the understanding, perceptions and lived experiences of young adults with genetic based visual impairment.

Method: Using qualitative research, fifteen participants were recruited, through purposive sampling, from various organisations for visually impaired individuals. In-depth interviews were conducted and analysed using thematic analysis.

Results: Many young adults identified the dilemma with regards to bearing children as the main implication of having a genetic based visual impairment. Almost all participants had minimal knowledge of their condition and felt that they did not understand it well. Many participants felt misunderstood by society. Social interactions were greatly impacted on by means of social alienation, support structures, and treatment by others. Having a disability created the sense of need and desire to succeed and prove themselves. An aspect that significantly impacted their lives was the challenges with mobility, which included the incapacity of driving and public transportation.

Conclusion: This research could assist in improving genetic counselling services, as well as provide organisations with information to strengthen support and guidance for these individuals.The study was funded by the NRF.

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P20 Psychological/Ethical/legal issues

P20.02B
A tremendous distress that goes unnoticed - the anxiety caused by abnormal results of Down syndrome screening

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Background: Screening tests for Down syndrome are routinely advised during pregnancy, with recommendation of invasive chromosomal testing to high-risk women (about 5% of pregnant population). The objective of our survey was to evaluate the anxiety experienced by women receiving abnormal screening results, and to explore potential ways to relieve this distress.

Methods: Women who had experienced an abnormal screening result for Down syndrome were invited to participate in an electronic anonymous cross-sectional survey. Anxiety level was evaluated by a six-item short-form of the Spielberger State-Trait Anxiety Inventory.

Results: Overall, 559 women have answered the questionnaire. In 86.04% high anxiety scores were reported following abnormal screening results. Using logistic regression analysis, higher anxiety scores were noted in younger women and in those informed of the abnormal result by the caregiver vs. written answer. 41.7% of the respondents preferred the risk reported as percentage (e.g. 1% risk for Down syndrome), 16.6% - as positive percentage (99% chance for fetus negative for Down syndrome), and only 4.4% stated they prefer the current form (e.g. 1 in 100). The participants noted several factors which could relieve their anxiety, including explanatory booklet or site (marked as "helpful" by 72.4% and 77.9% of the women, respectively).

Discussion: Women receiving abnormal results of Down syndrome screening experience significant anxiety, which seems to be underappreciated by the treating physicians. Efforts must be made to relieve this distress, including consideration of changing the historical report of the risk as a ratio to percentage or a combination of options.

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P20.03C
Fabry disease: Female perspectives

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Introduction: Fabry disease is a rare X-linked disorder caused by a mutation in the *GLA* gene. Women heterozygous for a mutation may have symptoms. Little is known about the psychosocial experiences of women heterozygous for Fabry disease. The aim of the study was to explore women's psychosocial experiences of being heterozygous for Fabry disease.

Materials and Methods: We used an explorative qualitative study design and conducted in-depth semi-structured interviews with ten Norwegian women who were known heterozygous for Fabry disease. We analyzed the interviews using inductive thematic analysis.

Results: Most women reported a relief of getting the Fabry diagnosis, as an explanation to present end previously unexplainable symptoms. It was however important for the women to make a distinction between Fabry disease and their daily life. The women on enzyme replacement treatment were grateful for available treatment; however, the treatment was not without personal cost. A frustration regarding the lack of health care providers' knowledge of Fabry disease was common.

Conclusions: Women who are heterozygous for Fabry disease react to and reflect on their diagnosis differently, and they should receive personalized information, support, and management. Health care providers should be aware and acknowledge feelings of guilt and difficulties in communicating about Fabry disease in the family. Health care providers should also recognize the personal cost of receiving treatment, and support the patients on this.

C. von der Lippe: D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; SHIRE, SANOFI. **J. Frich:** None. **A. Harris:** None. **K.N. Solbrække:** None.

P20.04D

Ethical decision-making competence regarding the possibilities of genome editing by CRISPR/Cas

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The CRISPR/Cas9 technology is proclaimed as one of the greatest innovations of the last decade and has sparked a public debate about its ethical issues. To facilitate participation of adolescents in public debates, the decision-making competence is fixed as a part of educational standards for the natural science curriculum in Germany. In the present study, we will analyze ethical decision-making competences of medical students and adolescents in the context of genome editing.

The Oldenburg model of ethical decision-making competences includes perception of moral relevance, assessment, judgement, argumentation, consequence reflection, change of perspective and basic ethical knowledge. On a paper-pencil test with open-ended questions on authentic ethical dilemmata, regarding genome editing for somatic or germline gene therapy for *RUNX1*-associated familial leukemia, the above-mentioned competences are recorded in a sample of 50 medical students and 50 adolescents of a secondary school. The responses of participants are analyzed using a structured qualitative content analysis. Points are awarded for the dimensions of ethical decision-making competence.

First results of the study show, that medical students and adolescents are open-minded towards genome editing in case of healing heavy diseases. Nevertheless, they have great fears about a misuse of this technology creating "designer babies" or "enhanced humans" and that human dignity might be hurt while tinkering in the DNA of embryos. None of them agree with the usage in general. The results of this study can be untilized to improve education on ethical decision-making competence and/or on basics about the CRISPR/Cas-based genome editing in school and university.

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P20.05A

Raw Genomic Data: Storage, Access and Sharing

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Whole Genome Sequencing (WGS) and Whole Exome sequencing (WES) for diagnostic and research purposes generate a large volume of raw data. Previous ethical and legal discussions concerning genomic data management have mainly focused on concerns related to interpretation of the results and the return of both primary and secondary findings from these tests. Yet to date, issues related to the storage and return of primary sequencing data (such as bam (Binary Alignment) or fastq files (unaligned reads)) that allow both the regeneration of primary results and the reanalysis of data have received far less attention, particularly in the clinical setting. This is despite the huge potential for long-term data storage to lead to future data re-analysis and reinterpretation in light of new evidence. In this paper, we critically appraise three main issues, namely, data storage policies and practices of clinical laboratories, patients' access to raw data, and sharing of raw data by individuals. We argue that there is a need for well-established and transparent raw genomic data retention and returning policies in order to enable patients to practice their rights to access raw genomic data. In addition, professional communities could guide laboratories in adopting best practices for the storage and return of raw data, and introduce uniformity to these practices. As WES and WGS become more embedded in diagnostics, it is timely to consider how current data storage policies align with patients' rights and interests to access raw data, and how these rights can be managed in the clinical setting.

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P20.06B

Incidental findings in genetic testing: a comparison between US and european guidelines and a bioethical reflection

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Next-Generation Sequencing (NGS) use in biomedical diagnosis and research makes now possible a deeper genetic and genomic analysis at a reduced cost. However, the analysis of evidence that is based on such big data can result in the generation of knowledge that is not relevant to the clinical question posed because some genes are associated with multiple medical conditions. These kind of information are commonly referred to as "incidental findings" and their management pose new ethical issues and dilemmas, especially regarding their disclosure to patients as well as the role of the different clinical figures involved. In this work we aim to analyze different recommendations and guidelines, referring in particular to USA and Europe. Numerous discussions have been conducted in order to reach a consensus on how to handle such findings in line with the legal and cultural particularities of individual states and general bio-ethical principles and different guidelines have been published. These reports mainly focus on the pros and cons of NGS technology and potential benefits and risks for reporting of incidental findings.We want to compare these various statements and try to figure out how patient's will could be better included in the decision making and the disclosure process, in order to respect its autonomy. We also point out the need for continued debate, research, and discussion among all stakeholders to improve our understanding of the effect that different policies have on patients, providers, laboratories, and societies.

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P20.07C
Consent model and return of results in oncological biobanks: a qualitative study

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Background: Biobanks provide biomedical research with high quality samples together with associated data including genetic data. Since the donors of biological material consent at the same time to storage and use of samples, to processing of personal data and to future research whose methods and aims cannot be clearly identified at moment of consent, there is currently no consensus on the best consent model. To benefit the donors one of the proposed ways is return of research results mainly from genetic research, which can reveal some of the future health risks. Current opinions differ in this regard taking into account practical but also ethical consideration like the "right not to know". The aim of this study is to explore the experience with and opinions on consent models and return of research results in practice of oncological biobanks.

Methods: Semi-structured interviews with the staff of 6 recently established oncological research biobanks in Czech Republic.

Results: In Czech oncological research biobanks the preferred model of consent is broad consent; none of the biobanks employs any interactive consent model („ unethical to remind someone about cancer"). Also return of research results was considered inappropriate for oncological patients („To whom the information should be communicated when the donor is dead?").

Conclusions: The recently established Czech research biobanks implemented in their practise broad consent and no return of research results mainly with regard to specific issues connected to oncological patients. This study was supported by project MŠMT-LM2015089 and RVO VFN 64165

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P20.08D
Moving towards clinical use of New Genome Sequencing technologies: Understanding the Ethical, Legal and psychological challenge

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Medical genetics illustrate the link between technological developments, an evolving scientific knowledge, and practical decisions which impact patients' lives. The rapid development of sequencing technologies marks a historical turning point in human genetics. Important questions need to be asked about *how to use these new technologies* in order to maximize the translational relevance of genetic research for patients and for society: How to give adequate information for patients in a context of uncertainty? How to deal with incidental/secondary findings? On the blurring between care and research? The introduction of emerging technologies in clinic requires technical/clinical validation, harmonisation of practice, and evolution of the legal and ethical framework. Drawing on our experience with the development of genomic technologies and their application in clinical settings, we analyze the ethical, legal and psychological issues inherent in the sharing, recording and storing of the medical information provided by these technologies. This approach takes into account the current context of genetic tests and provides concrete answers to the concerns of patients and professionals. Several issues to consider are identified: the status of large scale genetic information regarding privacy and confidentiality, clinically useful information, health related information where no immediate clinical measure exists. This work highlight the importance of a combined approaches of research in law, psychology and empirical ethic in order to describe the benefits and the limits of the use of new sequencing in clinics which will determine their ethical and social acceptability.

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P20.10B

Impact of the new EU *in vitro*diagnostics regulation on pharmacogemonics: the U-PGx project

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The Ubiquitous Pharmacogenomics (U-PGx) project aims at addressing the major challenges and obstacles to implement pharmacogenomics (PGx) testing in European patient routine medical care. In this context, U-PGx has to comply with the current European frameworks for performing genetic testing in practice notably the new *in vitro* diagnostic (IVD) medical devices regulation (IVDR). We will present in this paper the major changes induced by the adoption of several Regulations for the translation of PGx tests into clinics.

Since the adoption of the IVDR PGx tests and companion diagnostics (CDx) are now clearly falling under the scope of the Regulation (Class C according to the IVD's classification) with their respective definitions. Based on the evaluation of risks (from Class A, less, to D, high), this classification introduces some new rules regarding procedures. A notified body involvement is now required for the conformity assessment procedure, with specific provisions for CDx. The performance study for marketing new IVDs should be based on the clinical benefit of the device (scientific validity, analytical performance and clinical performance). New traceability and transparency rules are also provided, such as the Unique Device Identification system and a summary of safety and performance. Moreover, manufacturers have to put in place surveillance and vigilance systems with the obligation to produce several reports.

Additionally, as for the General Data Protection Regulation, a person responsible for regulatory compliance must be present.

And finally, as for clinical trial data, a new database is introduced: Eudamed, including several databases on medical devices and IVDs.

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P20.11C

Precision medicine and its infrastructures of solidarity. Probing the social contract in US and EU precision medicine initiatives

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On a global scale, a 'race for innovation' between nations has begun to set off for the building of Precision Medicine (PM) initiatives. To establish PM, various initiatives call on 'a new social contract' between citizens and the health care system. This contract implies that citizens recognize that they and everybody else will benefit from medical science if they allow data about their own genome to be collected and shared. Policy makers thereby mark PM not just with grand claims on scientific and economic benefits, but also with the formation of a new genomic citizenship. In this paper, we investigate the mobilization of the social contract in the economic competition between 'PM nations'. Drawing on a comparative analysis of the United States and Europe, we show how these new social contracts for PM are not only

imagined as a critical part of the successful development of PM, but are materialized in infrastructures of health care delivery. We demonstrate how the successfulness of PM depends on infrastructures of health care delivery that perform as trust-generating techniques for the mutualization of benefits and rights. We consider the role of infrastructures of health care delivery, which we coin as "Infrastructures of Solidarity", as key markers for the implications of efforts to incorporate Precision Medicine (PM) in contemporary health care systems. We conclude by a reflection on what the new social contracts for PM entail for conditions of citizenship, access to health care, and the future of the welfare state.

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P20.13A

Population biobank participants' preferences and response to disclosure of unexpected genetic findings

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Introduction: The Estonian biobank legislation gives participants the right to receive results. Disclosure of unexpected genetic findings to population biobank participants through genotype-first approach provides valuable information on how individuals from the general population would respond.

Methods: A project involving participants carrying pathogenic variants in BRCA1 and 2 genes associated with familial breast and ovarian cancer (HBOC) was initiated. The procedural framework includes contacting without disclosing genetic status, project specific consent, independent validation, disclosure with genetic counseling, collaboration with oncologists, and cascade screening. Immediate and long-term follow-up were surveyed to understand the psychological and behavioral impact of results disclosed.

Results: Of 49 carriers identified 22 have visited the biobank and expressed interest towards return of results. Of these participants, only three had no known family history of HBOC, and one individual had previously received genetics evaluation. All 19 participants who received results perceived this information valuable. Although three participants reported feeling worry at first, these feelings did not remain long-term. According to 6-month follow-up all 12 responders cope with the finding, none regret the decision made, and all communicated the finding to their physician and family. Nine of 12 have seen a specialist and five pursued risk-reducing surgery.

Conclusions: The genotype-first approach demonstrated that currently only a minority of individuals carrying pathogenic BRCA mutations are under appropriate medical surveillance. Although the 45 percent response-rate supports choice regarding disclosure, all participants perceived results received valuable. Long-term follow-up showed that information is shared generously and demonstrated increase in screening and prevention behavior.

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P20.14B

Secondary findings from whole exome or genome sequencing: psychological and ethical Issues. Patient point of view

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Introduction: Discovery of secondary findings (SF) in diagnostic practice is a major psychological and ethical issue for the introduction of genomic medicine.

Materials and Methods: The purpose of this qualitative study is to analyze patient expectations for SF and to

identify the reporting procedures necessary for informed consent, by interrogating patients with no diagnosis, but also patients facing diseases concerned by SF. Five focus groups (FG) were led by psychologists: FG1 (n=5) without diagnosis; FG2 (n=10) oncogenetics; FG3 (n=3) cardiogenetics; FG4 (n=3) metabolic diseases; FG5 (n=4) heterozygotes for frequent recessive diseases. Five questions were asked regarding access to SF: 1. Favorable or not. 2. Opinion concerning minors. 3. Concerning equity of access. 4. Concerning reporting procedures. 5. To FG5 only: opinion on the search for heterozygosity for frequent recessive diseases. The discussions were recorded and then analyzed with qualitative research methods.

Results: Overall, 10/25 were favorable to the actionability of SF, but 13/25 unfavorable because of the psychological consequences. 9/25 modified their views over the course of the discussions. 60% were favorable and 30% ambivalent to the access of SF by minors. Equity of access to SF led to philosophical discussions on quality of life. The 4 key information-based issues for patients were: explanation of the issues of SF, the importance of a reflection period, autonomy of choice, and quality of information.

Conclusions: The weight of individual experience was decisive in patient expectations and fears regarding access to SF. Longitudinal studies based on real announcements are necessary to establish guidelines.

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P20.15C

Cognitive profile in Silver-Russell syndrome: a first French study in adults

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Introduction: Silver-Russell syndrome (SRS) is a rare disorder characterized by severe intrauterine and postnatal growth retardation with relative macrocephaly at birth, typical dysmorphic features, and feeding difficulties. In the majority of cases, SRS is caused by a methylation defect of 11p15 imprinting center region 1. To our knowledge, only three studies have documented the cognitive development of children with SRS, but these are old studies in which patients were diagnosed only by non-standardized clinical criteria. Currently, no studies have been published on the assessment of the intellectual functioning of adults.

Materials and Methods: An evaluation of intellectual efficiency was performed in 8 patients with SRS (4 males, 4 females), aged 20 to 39 years (mean age 23.75 years) followed at France from 2016 to 2018. All patients had an epimutation of the region 11p15.

Results: Mean overall IQ score was 89.13 (14.33 SD) with a range between 71-107. Verbal comprehension index was on average higher than other indexes (mean 104.50, 18.55 SD). 50% were treated with growth hormone. Speech delay in childhood and learning difficulties have been reported in some patients. 75% have benefited from speech therapy and/or psychological care. Seven patients had a bachelor's degree and five patients completed higher education.

Conclusions: Overall adults with SRS had normal intellectual efficiency and a fairly positive life course. If more patients to study are needed to objectify these results, they can improve knowledge about SRS and point to the importance of early intervention and multidisciplinary care from childhood to adulthood in SRS.

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P20.16D

Attitudes towards reproductive carrier screening among the genetic muscle disorder community

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Methods: We conducted a population-based study of the prevalence and outcomes of genetic muscle disorders in New Zealand. All those diagnosed on or before the point prevalence date of 1st April 2015 were included. All eligible cases were invited to participate in an assessment to determine the impact of the disorders on people's lives, including their attitude towards preconception screening for genetic muscle disorders.

Results: 596 participated in the impact assessment, 83 of these were a parent of an affected child(ren). The majority (87%) of those with a disorder were in favour of reproductive carrier screening for their disorder as were parents of an affected child (84%). Parents of older children (11 - 15 years) were less likely to be in favour of preconception screening than parents of younger children (74% vs 92%). People diagnosed with a less severe disorder, such as *myotonia congenita*, were more likely to not favour preconception screening compared to those diagnosed with a more severe condition. All diagnosed with Duchenne's favoured screening.

Conclusion: The majority of people living with genetic muscle disorders are in favour of reproductive carrier screening for that disorder. By examining sub groups within the overall cohort (age, severity, inheritance pattern), we are able to get a more detailed and nuanced view of attitudes towards reproductive carrier screening. An understanding of what the community want with respect to the accessibility and acceptability of screening is important when considering utilising new genetic technologies to offer the option of screening individuals to identify carrier status for genetic disorders.

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