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ABSTRACTS COLLECTION

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Posters

P01
Reproductive Genetics/Prenatal Genetics

P01.01A
Prenatal whole exome sequencing in agenesis of the corpus callosum

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Agenesis of the corpus callosum (ACC) is usually diagnosed by prenatal ultrasound examination. In case of isolated ACC (iACC), neurodevelopment is within normal range in 80 % of cases whereas 20 % of children present mild to severe intellectual disability (ID). Among genetic etiologies, only chromosomal causes are investigated (karyotyping and microarray) during prenatal period while ACC with ID is due to a single gene mutation in most cases.

Thus, parents make the decision to continue or terminate the pregnancy on statistics.

Our study aims to evaluate the feasibility of prenatal testing of all known ACC genes using whole exome sequencing (WES).

Eighteen fetuses with ACC were included (ongoing inclusions): 14 iACC and 4 cases of ACC associated to other anomalies (aACC). Trio WES were performed on fetal DNA extracted from amniotic fluid sampling. Only pathogenic variants in known ACC genes were considered. Variants of unknown significance (VUS) and secondary findings were not reported. WES results were available within an average of 21 days. Thirteen WES (72%) were normal (11 iACC, 2 aACC). A pathogenic variant in an ACC with ID gene was identified in two cases (11%): BRAT1 (aACC) and PPP2R1A (iACC). A pathogenic 6q27 deletion was identified in one case (aACC). We identified a VUS in 2 cases.

Our preliminary results suggest feasibility of prenatal WES in fetuses with prenatal diagnosis of ACC. In case of etiological diagnosis, WES helps the parents to make a decision for the pregnancy. However, WES in the prenatal period arises ethical questions.

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P01.02B

Results of karyotype analysis of 8361 pregnancies in prenataly identified cases with amniocentesis from south of Turkey

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Objectives: Amniocentesis is a very crucial diagnostic procedure for preventing the birth of genetically defective fetuses in order to decrease the prevalence of genetic diseases in populations. Methods: A retrospective review of our amniocentesis database for the period from January 2000 to February 2019 was carried out. The karyotyping of 8361 fetuses was carried out in Department of Medical Biology from the samples of amniotic fluids which were sent from Department of Obstetrics and Gynecology of Balcali Hospital. A standard nomenclature has been developed to describe each of types of abnormality found in human chromosomes.

Results: A total of 8361 amniocentesis specimens were processed during the study period. 601 fetuses (7.18%) had various chromosomal abnormalities. 54.4% of abnormal karyotypes (329 cases) were numerical and 43.09% (259 cases) were structural. Both numerical and structural chromosomal aberrations were observed in 13 cases (2.16%). The ratios were as: trisomy 21 (48.93%), trisomy 18 (17.93%), monosomy X (9.72%), trisomy 13 (6.99%), Triploidy (4.86%), Klinefelter Syndrome (3.34%), Trisomy X (1.21%), XYY Syndrome (0.91%), and the others in all numerical abnormalities. The frequent structural abnormalities were as: 46,XX/XY, inv(9) (p11;q12)/(p11;q13) (29.34%), 46,XX/XY, 1qh(+)(11.58%), 46,XY, Yqh(-) (7.33%), 46,XX/XY, 16qh(+)(7.33%), 46,XX/XY, 9qh(+) (4.63%) and 46,XY, Yqh(+)(4.24%). Balanced and unbalanced translocations, deletions and duplications were also found in less ratio.

Conclusions: According to the literature and our results, advanced maternal age is the main cause of fetal chromosomal abnormalities. Fetal chromosomal abnormality ratio that we found was 7.18%. This ratio emphasizes the importance of prenatal diagnosis.

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P01.03C

Biallelic variants in AMPD2 gene cause prenatal isolated agenesis of corpus callosum

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Introduction: Recessive mutations in *AMPD2* gene cause Pontocerebellar Hypoplasia (PCH) type 9. This severe

neurodevelopmental disease is characterized by intellectual disability associated with epilepsy and spasticity. Brain imaging shows the association of pontocerebellar anomalies, agenesis of corpus callosum (ACC) and microcephaly. The 2 fetuses already reported with mutations in this gene had HPC associated with ACC on prenatal ultrasound or MRI. We report two cases of AMPD2 mutations in sibling fetuses with prenatal diagnosis of isolated ACC (iACC).

Cases: Male fetus 1 : prenatal iACC at 32 gestational weeks (GW) ; termination of pregnancy (top) at 37GW, normal CGH array; postnatal iACC (neuropathological examination). Male fetus 2: prenatal iACC ; top at 31 GW; normal CGH array; postnatal iACC (neuropathological examination).

Method: Whole exome sequencing (2 fetuses and 2 parents).

Results: Identification of two compound heterozygous *AMPD2* mutations (c.751C>T, p.Arg251Trp; c.1648G>A, p.Glu550Lys) in the 2 fetuses.

Discussion: We report here an unusual prenatal presentation of PCH9 in 2 sibling fetuses, limited to a prenatal and postnatal iACC. *ACC* is one of the most frequent brain malformations, usually detected by prenatal ultrasound. Prognosis is variable and correlates with the presence or absence of other malformations. When isolated, the neurodevelopment is within a normal range in 80 % of cases whereas 20 % have mild to severe intellectual disability. Establishing an etiological diagnosis of a prenatal apparently iACC is essential to assess neurodevelopmental prognosis. *AMPD2* should be considered as a gene responsible for isolated prenatal ACC.

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P01.04D
Rare autosomal abnormalities detected using noninvasive prenatal screening

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Introduction: Noninvasive prenatal DNA screening (NIPS) is believed to be highly sensitive to common aneuploidy detection. Since some NIPS technologies use whole genome sequencing, aneuploidies and large CNV of any chromosome could be detected. Although detection of rare aneuploidies and large CNV is possible it is not recommended in Obstetricians and Gynecologists guidelines. Majority of NIPS providers do not report this information.

Materials and Methods: NIPS was carried on Ion S5 sequencer with in-house developed data analysis pipeline. 1300 samples collected at 11-20 weeks of gestation were analyzed. NIPS results were confirmed with karyotyping of invasively obtained samples or blood cells obtained postnatally. Four biopsies of placental tissue were performed to assess placental karyotype.

Results: High aneuploidy risk was detected in 39 cases. 14 - trisomy 21, 3 - trisomy 13, 15 sex chromosome abnormalities (4 due to maternal mosaicism) and 7 rare autosomal abnormalities (trisomies 7, 8, 10, large CNV). In one case large CNV was confirmed in the fetus. In two cases of rare trisomies, placental tissue was available, in both cases, mosaic trisomy was confirmed in the placenta.

Conclusions: High risk of rare autosomal abnormality could be associated with miscarriage, confined placental mosaicism, true fetal mosaicism, and uniparental disomies. Whole genome analysis may identify pregnancies at risk of miscarriage and other complications.The study is supported by the Ministry of Healthcare of Russian Federation

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P01.05A
Array CGH and next-generations sequencing in diagnostics of fetal skeletal dysplasia

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Background: Array CGH is routinely used in prenatal diagnostics of structural fetal defects where the possibility to find genomic aberrations is high. This technique is especially suitable in a history of repeated pregnancy failure together with structural defects.

Materials and Methods: We report here the results from genomic analysis in fetal skeletal dysplasia (SD) - we performed arrayCGH in cases with repeated abortions and fetal skeletal anomalies and next-generation sequencing (NGS) -

in cases with lack of diagnosis after aCGH or phenotypes, strongly suggestive for monogenic disorder.

Results: The findings from arrayCGH and NGS analyses are presented in the Table below.

Clinical manifestation	Genomic/gene aberration
Limbs anomalies and 3 previous abortions	7q21.3(94,214,445 - 95,257,297)x1 (1.04Mb) Split hand/foot malformation
3rd pregnancy with phocomely and limbs aplasia	1q21.1 (145425395 -146507577)x1 (1.08 Mbp) TAR syndrome
Skeletal dysplasia and 2 previous abortions	17q21.33(48,264,999-48,277,988)x3 (12.99 Kbp, including *COL1A1* gene)
2nd pregnancy with arthrogryposis	Xq28(154,974,667-155,208,354)x0 (234 Kbp) VOUS: c.357T>G, p. Ile119Met (NM_017946.3) in *FKBP14* gene
1st pregnancy with dwarfism	Possibly damaging variants: c.6117C>G (p.Ile2038Met) in *HSPG2* gene; c.7223C>G (p.Ala2408Gly) in *PCNT* gene

Conclusion: Genetic diagnosis of SDs is strongly needed because there are so many diseases with complex phenotypes and identification of the responsible gene(s) is important to understand the diseases themselves. We demonstrated here the diagnostic utility of aCGH, which could be the first-line test in prenatal settings. NGS is a very powerful technique in diagnostics of very rare diseases, but needs much longer time for analysis and additional steps for elucidations of the found variants.

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P01.06B
Application of array comparative genomic hybridization (aCGH) for identification of lethal chromosomal aberrations in spontaneous abortion

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Spontaneous abortions occur in 8-20% cases of recognized pregnancies. Most often, the miscarriage take place in the first trimester (7-11 weeks). There are many causes of pregnancy loss, but the most important (~75%) is the presence of chromosomal aberrations. A high percentage of chromosomal aberration is caused by the abnormal genetic material of germ cells. Research showed that 20-30% of ova and 6-8% of sperm showed chromosomal abnormalities. This percentage significantly increases with advancing age in women and in cases of abnormalities of sperm in men.

We present the results of oligonucleotide array application in a cohort of 31 cases of mothers with adverse pregnancy histories. DNA was extracted from trophoblast and umbilical cord. Array CGH was performed using 4x180K microarrays from Oxford Gene Technology (CytoSure ISCA, v3).

The detection rate in our cohort was 61,3% (19/31). The most commonly found (73,7%) were aneuploidies: trisomy of chromosomes 14, 16, 18, 21, 22, Turner syndrome and triploidy. Other chromosomal abnormalities included structural aberrations: deletion 7p22.3p12.3 and duplication 9p24.3p13.2 inherited from normal father, deletion 3q13.31q22.2, deletion 3q22.3q23, duplication 17p12 inherited from father with foot malformations, deletion 17p13.1 inherited from normal mother, deletion 5q14.3 and *de novo* deletion 1q21.1q21.2.

Our research shows that microarray is the only method permitting the identification of all unbalanced aberrations (number and structure) with a much higher resolution than karyotype.

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P01.08D
Screening by Multiple ligation-dependent probe amplification assay of AZF a, b, c regions in primary infertile men with azoospermia

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Y chromosome microdeletions are the most common cause of azoospermia and occur in 5-15% of infertile men with azoospermia. Microdeletions/partial deletions of the azoospermia factor(AZF) regions on the Y chromosome are a well-known genetic cause of male infertility, resulting in impairment of spermatogenesis. We sought to determine the frequency and the character of AZF region microdeletions/partial deletions in infertile men with azoospermia by using

Multiplex Ligation-Related Probe Amplification (MLPA). In total, 50 azoospermic infertile men without gene and chromosome mutations, were screened for Y chromosome microdeletions in AZF regions and 50 fertile men assigned to control group. The total frequency of the microdeletions was 16%. Most deletions (10%) were seen in the AZFc followed by the AZFb (4%). The partial BPY2 gene deletions, located at AZFc region were detected in five patients while partial EIF1AY gene deletions, located in AZFb locus were seen in two patients. The combined AZFb and AZFc loci deletion was revealed in a patient. No AZFa region deletion was detected among the azoospermic cases. In the literature, it has been shown that BPY2 gene is effective in male germ cell development and loss of EIF1AY gene function can result in azoospermia sporadically. The present findings suggest that MLPA is more appropriate compared to mPCR because it investigates partial and complete deletions of AZF regions for male sterility compared to previous methods. Our study shows that microdeletion and partial deletions, which are mentioned in the literature as the reason of azoospermia, can be detected by MLPA method at the same time.

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P01.09A

Characterisation of Bardet Biedl Syndrome in a foetus by post-mortem microfocus computed tomography

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Confirmation of antenatally detected foetal anomalies is vital following termination of pregnancy due to congenital anomalies. Phenotypic information is essential for genetic testing and counselling. However antenatal ultrasonography identifies only up to 68% of autopsy findings, which are not always available.

Post-mortem imaging using microfocus computed tomography (micro-CT) provides an alternative, or complementary tool, to autopsy. It provides high-resolution, 3D digital images, which may enable accurate diagnosis of complex multisystem syndromes, in particular in small early gestation fetuses.

We describe the case of a 33 year old primigravida, who attended for antenatal sonography at 12^{+5} weeks gestation. An increased nuchal translucency and hypoplastic left heart were observed; multicystic kidneys suspected. Array-CGH was normal. The couple opted to terminate the pregnancy at 15^{+6} weeks.

Due to small foetal size, whole-body micro-CT was performed. Image analysis revealed an atrioventricular septal defect, mitral valve stenosis, left ventricular hypoplasia, aortic atresia and ascending and aortic arch hypoplasia. Multiple renal cortical cysts and bilateral upper and lower limb post-axial polydactyly were observed. Renal histology confirmed extensive cystic dilatation of the renal tubules.

Genetic testing identified compound heterozygous mutations in *BBS7* [c.187G>A; p.(Gly63Arg)mat and c.973G>T; p.(Glu325Ter)pat], consistent with Bardet-Biedl Syndrome. Given a recurrence risk for future pregnancies at 25%, the couple received counselling regarding future preimplantation genetic testing.

In conclusion, micro-CT is a non-invasive highly detailed method for assessing early gestation foetal anatomy, and provides an adjunctive or alternative tool to the standard foetal autopsy technique.

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P01.11C

Preconception expanded carrier screening in a developing country: where does science exactly meet bioethics?

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Introduction: Expanded carrier screening (ECS) was recently introduced to Thailand and other developing

countries in Asia by a large number of international laboratories. Several incidences were reported concerning negative psychosocial impact, i.e., anxiety and difficulty in making decision on reproductive option.

Material and Methods: Forty healthy Thai couples visiting preconception clinic were enrolled. ECS was performed using 630-disease panel covering most autosomal recessive and X-linked disorders. Thereafter, attitudes on ECS and clinical application were interviewed. Genetic data were then combined with previous in-house database, pooling up to 355 unrelated individuals. Carrier identification were interpreted using our developed bioinformatic and clinical criteria suitable for legal and ethical issues.

Results: The most common carrier frequency in our cohort belonged to beta thalassemia (1/5) which was consistent with national statistics. Interestingly, primary hemochromatosis H63D, which have never been recognised in Southeast Asian populations, became the second common (1/16). Other carriers detected as common in Thais included alpha thalassemia, Gilbert syndrome, 21-hydroxylase-deficient congenital adrenal hyperplasia and spinal muscular atrophy. More than 95% of the subjects had a positive attitude to ECS. They accepted preimplantation genetic diagnosis as their reproductive option if they were identified as couples at risk, either mild or severe condition.

Conclusions: ECS would be useful if the selection criteria were clear and consistent with national regulation and ethics. Pre-test genetic counseling was necessarily required for the couples to understand further outcome.

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P01.12D
Interest in expanded carrier screening among prospective parents: systematic review of the literature

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Expanded carrier screening (ECS) is a reproductive genetic test aimed at identifying prospective healthy parents who are at risk of conceiving a child affected with a recessive disorder. ECS tests screen for large numbers of recessive disorders and are currently available through various genetic testing laboratories. However, the extent to which ECS can be successfully implemented in the context of reproductive healthcare depends largely on prospective parents' interest in ECS. In this systematic review, we synthesized evidence from empirical studies exploring interest in ECS among individuals and couples in the general population. Studies included in this review reported prospective parents' intentions to undergo a (hypothetical) ECS test, uptake of an actual ECS offer, or both. Four databases (Pubmed, Web of Science, CINAHL, Cochrane Library) were systematically searched for relevant publications and 12 empirical studies were included in the review. Owing to the novelty of ECS, all the included studies were relatively recent, having been published during 2015-2019. In the included studies, 33%-66% of respondents were interested in a (hypothetical) ECS test, while uptake rates for actual ECS offers were reported at 10%-50%. The highest uptake was observed in a study where ECS was offered to pregnant women. By contrast, studies focusing on the preconception population reported lower uptake rates, even though some of these preconceptional ECS tests were provided free of charge. Our findings suggest that there may be discrepancies between prospective parents' self-reported intentions to undergo ECS and their actual test-taking behavior, particularly during the preconception period.

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P01.14B
Prenatal clubfoot increases the risk for clinically significant chromosomal microarray (CMA) results - analysis of 269 singleton pregnancies

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Clubfoot, also known as talipes equinovarus, is a well-recognized congenital foot deformity. It is diagnosed in about 1:1000 pregnancies. Clubfoot can present as an isolated defect or as non-isolated, also called "complex" or "syndromic", clubfoot, associated with additional anomalies.

Objective: This work was done in order to examine the detection rate of clinically significant chromosomal

microarray analysis (CMA) results in singleton pregnancies with clubfoot.

Methods: Data from all CMA tests performed due to sonographic abnormal findings between January 2013 and September 2017 were retrospectively obtained from Ministry of Health computerized database. All singleton pregnancies with sonographic diagnosis of clubfoot (talipes equinovarus) and documentation of CMA result were included.

Results: Of the 5750 CMA tests, a total of 269 (4.7%) were performed due to demonstration of fetal clubfoot. Of the 229 cases with isolated deformity, nine (3.9%) clinically significant CMA results were detected. This detection rate is significantly increased compared CMA results in normal pregnancies as previously reported. In 40 pregnancies with syndromic clubfoot, seven (17.5%) clinically significant CMA results were detected, a significantly higher frequency compared to isolated clubfoot cases.

Conclusion: Sonographic diagnosis of clubfoot, whether isolated or associated with additional sonographic anomalies, seems to increase the risk for abnormal CMA findings. Thus, CMA analysis, in conjunction with thorough sonographic anatomic survey, should be recommended in such pregnancies.

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P01.15C
The influence of prenatal chromosomal microarray analysis on the decision on termination of pregnancy

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Introduction: Chromosomal microarray analysis (CMA) was introduced to prenatal genetic diagnosis a couple of years ago. There have been many publications before demonstrating the benefit of CMA in detecting microduplications and microdeletions in the prenatal setting. Aim of this study is to evaluate the association between prenatal CMA and the decision on termination of pregnancy.

Materials and Methods: This is a retrospective study of a single prenatal center in Austria between 2011 and 2017, including all fetuses that had prenatal diagnosis with CMA.

Results: In general highest rates of pathogenic CNVs were found if the indication for prenatal diagnosis was congenital heart disease or brain malformations. There was a statistical significant association between pathogenic CNV and the decision on termination of pregnancy ($p<0,05$). But comparing pregnancy outcomes with prenatal CMA and without CMA, irrespective of the result of the CMA, we found significant fewer terminations of pregnancies in the group with CMA, even if the severity of malformations of the fetuses were comparable.

Conclusions: In summary the decision on termination of pregnancy is individual and there are many influencing factors to consider. However our data suggests that the result of additional CMA can be a crucial factor in the decision on termination of pregnancy.

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P01.16D
Congenital anomalies in children born after assisted reproduction in the Czech Republic: Population based study

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Introduction: Assisted reproduction (AR) is nowadays commonly-used method for treating various fertility problems. However, several studies have shown a higher incidence of selected types of congenital anomalies among AR-conceived children. Our goal was to study this association on a large population cohorts (over 3 millions of live-births) using data from our population based registries.

Methods: Our retrospective epidemiological study is based on the official data from the National Registry of Congenital Anomalies and National Registry of assisted Reproduction (run by the Institute of the Health Information and Statistics of the Czech Republic). The registration process is population-wide and compulsory by national law. We evaluated the incidence of congenital anomalies (ICD-10 diagnoses Q00-Q99) in AR-conceived children and compared it to the incidence of congenital anomalies in naturally-conceived children. Time period: 2013-2015.

Results: The overall incidence of congenital anomalies was slightly higher in the AR-children group however, the difference was not statistically significant. The congenital anomalies were more common in twins ($p<0.005$). The incidence of congenital anomalies in AR-twins was significantly higher than the incidence of congenital anomalies in non-AR-twins ($p<0.005$) while the difference of congenital anomalies incidence in AR and non-AR singletons was not significant.

Discussion: We have found that the incidence of congenital anomalies was higher especially in twins born after assisted reproduction while in singletons the difference was not significant. We will further analyze this finding during the next phases of our population study.

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P01.17A

Carriers for deafness and vision loss are identified as common autosomal recessive conditions in 40 healthy Thai couples obtaining expanded carrier screening

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Introduction: One of the applications for next-generation sequencing (NGS) is expanded carrier screening (ECS) which offer prospective parents to learn their carrier status of many autosomal recessive (AR) and x-linked recessive (XLR) diseases at once. However, designing an ECS panel for Thais can be difficult due to insufficient variant frequency data. Therefore, we conduct a study to gather the frequency of pathogenic variants in the associated diseases by offering ECS to a Thai couple who plan to conceive.

Methods: DNA samples of 40 non-consanguineous Thai couples were underwent sequencing by NGS in 540 genes associated with AR and XLR diseases. We classified the variants detected by NGS using population databases (1000 Genomes Project, gnomAD, and our in-house Thai exome database) and diseases databases (Clinvar and HGMD professional) to determine their pathogenicity.

Results: 76 variants with strong evidence for pathogenicity are identified. Deafness is the most common AR diseases with six individuals carry deafness-related variants in these genes: *SLC26A4*, *USH2A*, *OTOF*, and *MYO3A* followed by five carriers of vision-loss variants in *GRM6, USH2A, EYS*, and *ABCA4* gene. One couple shared a different pathogenic mutation in *the ABCA4* gene.

Conclusions: Though deafness and vision-loss are the two most common AR carrier found in the Thai population, consideration need to be taken if they were in the ECS panel because these phenotypes are not severe enough for a prenatal diagnosis.

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P01.18B

High risk - what's next? Decisional conflict, regret and satisfaction among pregnant women making choices about further prenatal testing after a high probability result from the combined test

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Introduction: The aim was to investigate decision-making among Danish high-risk pregnant women when choosing between non-invasive prenatal testing (NIPT), invasive testing or no further testing. Women with a high-risk result from the combined first trimester screening were invited to fill in two online questionnaires at GA 12-14(Q1) and GA 24(Q2). The scales used were Decisional Conflict and Regret Scales, Satisfaction with genetic Counselling Scale and Health-Relevant Personality Inventory.

Results: In total, 339 women were included, and the response rates were 76 % on Q1 and 88% on Q2, respectively. Of the participants, 75.4% chose an invasive test and 23.8% chose NIPT. The median DCS score among all participants was within the level associated with implementing decisions, whereas 13.3% had a high level of decisional conflict. Choosing NIPT was associated with a high decisional conflict; receiving genetic counselling the same day was associated with a high decisional conflict; and a high satisfaction with the genetic counselling was associated with low decisional conflict. Furthermore, 'alexithymia', the personality sub-trait that describes a disinterest or inability in identifying and understanding feelings, was associated with low decisional conflict. High decisional regret was associated with high decisional conflict and low satisfaction with genetic counselling.

Conclusion: The results from this study show that satisfaction with, and timing of counselling are essential factors to limit decisional conflict. Also, the results indicate that women choosing NIPT have more decisional conflict compared to women choosing invasive testing. There was a significant association between high decisional conflict and later decisional regret.

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P01.19C

A novel diagnostic panel of 15 STR markers used to confirm the detection of Duchene Muscular Dystrophy, aneuploidy screening, and sample authenticity testing

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Introduction: Duchenne (DMD) and Becker muscular dystrophy (BMD) are one of the most common genetic muscular dystrophies. Prenatal diagnosis (PND) or Preimplantation Genetic Diagnosis (PGD) could be attractive options to prevent the birth of new affected cases. Aneuploidies are the cause of over 50% of all miscarriages. Early aneuploidy screening in conjunction with PND or PGD can decrease the subsequent complication of pregnancy termination.

Methods: This study aimed to develop a novel panel to confirm detection of DMD/BMD and aneuploidy screening simultaneously. The panel functions on the principle of hemizygosity mapping of the 6 novels STR (Short Tandem Repeat) markers linked to dystrophin gene. Additionally, this includes autosomal STR markers for the critical regions of Chromosomes 21, 18, 13, X and Y. These markers were amplified in a time-saving and cost-effective multiplex PCR reaction.

Results: Allele frequency and heterozygosity assessment of STR markers were studied in 250 unrelated healthy individuals. Totally, 85 alleles were detected. Heterozygosity of markers was 75.7%-89.8%. Genotype frequencies of markers were found to be in agreement with the Hardy–Weinberg equilibrium ($P \geq 0.1899$). For further confirmation direct mutation analysis was also performed. The results were compatible. The panel was used for more than 150 PND cases and 15 PGD candidates and the results were successful.

Conclusion: This panel increases the accuracy and sensitivity of diagnosis. It can be easily applied for PGD/PND of DMD, aneuploidy screening and sex determination. Also provides extra advantages like ruling out maternal cell contamination, paternity testing and avoidance of sample cross-contamination.

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P01.20D

Relative telomere length in women with early pregnancy losses

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Introduction: Early pregnancy loss (EPL), a spontaneous abortion before 14 weeks of gestation, occurs in ~ 15% of clinically-recognized pregnancies and is the most common complication of pregnancy. Recurrent early pregnancy loss (REPL) affects 1-2% of couples and has a complex etiology. Approximately half of miscarriages from EPL cases are caused by chromosomal abnormalities in the embryo. Spontaneously lost pregnancies are characterized by shortened telomeres, especially in embryos with aneuploidies. Thus, short telomere length may be more frequent in women with REPL.

Material and Methods: Relative telomere length (RTL) was measured in DNA isolated from the blood samples using a real-time polymerase chain reaction approach. RTL was examined in three groups of women with EPL (N=171): (i) patients with single EPL (EPL1) (N=52), (ii) patients with two EPL (EPL2) (N=68), (iii) patients with REPL (N=49); and control group (C) - women who had healthy pregnancies with no history of infertility or miscarriage (N=113).

Results: The EPL group had significantly lower RTL than control (EPL: 1.39±0.06 versus C: 2.23±0.01, P=0.0000001). Average RTL were similar in EPL2 and REPL groups (1.36 ±0.04 in EPL2 and 1.31±0.07 in RPL) and were significantly lower compared to control (EPL2: 1.36 ±0.04 vs C: 2.23±0.01, P=0.000001 and REPL: 1.31 ±0.07 vs C: 2.23±0.01, P=0.000001).

Conclusions: Women experiencing two or more EPL have shorter telomeres. We assume that short telomeres in women are involved in complex factors that provoke early pregnancy loss.

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P01.21A

Endometrial receptivity revisited: endometrial transcriptome adjusted for tissue cellular heterogeneity

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Introduction: The proportions of epithelial and stromal cells vary in endometrial tissue during the menstrual cycle. Each cell type has its unique gene expression profile, that compose whole-tissue expression pattern.

Materials and Methods: Using cell-type specific transcriptome data and computational deconvolution approach, we estimated the epithelial and stromal cell proportions in whole-tissue biopsies taken during early secretory (ES) and mid-secretory (MS) phases. The estimated proportions were used as covariates in whole-tissue differential gene expression (DGE) analysis. For RNA sequencing we used paired ES and MS endometrial biopsies, obtained from 35 healthy, fertile volunteers (23 - 36 years). DGE analysis was performed using two approaches - with and without deconvolution step, and results compared with each other.

Results: The estimated average proportions of stromal and epithelial cells in ES phase were 65% and 35%, and during MS phase 46% and 54%, that correlated with histological evaluation (r=0.88, p=1.1×10-6). Endometrial DGE analysis showed 26% differentially expressed transcripts (n=946) in receptive endometrium in both cell-type unadjusted and adjusted analyses. However, the other 74% (n=2,645) become statistically non-significant after cell-type adjustment, underlining the impact of tissue heterogeneity on DGE analysis. The results suggest new mechanisms involved in endometrial maturation involving genes like LINC01320, SLC8A1 and GGTA1P, described for the first time in endometrial receptivity context.

Conclusion: The better understanding of molecular processes during transition from pre-receptive to receptive endometrium serves to improve the effectiveness of assisted reproduction protocols. Biopsy cellular composition should be taken into account in future endometrial 'omics' studies.

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P01.22B

A prospective evaluation of exome sequencing in 51 fetuses with multiple congenital anomalies and lessons for future prenatal implementation

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Introduction: Exome sequencing (ES) is a powerful adjunct to post-mortem examination for investigating fetuses with congenital anomalies. However, with the implementation of rapid "in-utero" sequencing becoming eminently feasible, fetal phenotyping may, in future, rely on antenatal imaging alone.

Aim: To determine whether the diagnostic yield of ES in fetuses with congenital anomalies following autopsy is comparable to the yield of ES implemented at the time of antenatal ultrasound detection.

Method: ES was prospectively performed on 51 fetal probands following autopsy as part of the Perinatal Autopsy Flagship of the Melbourne Genomics Health Alliance Demonstration Project. Candidate variants concordant with phenotypic findings from fetal autopsy were classified by multidisciplinary review using ACMG guidelines. Independently, a phenotype-driven virtual gene panel for each proband was derived from antenatal imaging reports by clinicians blinded to autopsy findings. Where a pathogenic variant fell within this "pre-autopsy" virtual gene panel, this was viewed as a diagnosis that would have been made by combining WES with antenatal imaging alone.

Results: The diagnostic rate in this cohort was 17/51 (33%) with 4 further variants awaiting functional validation. Phenotypes with the highest diagnostic yield included skeletal dysplasias (100%); multiple malformations (33%) and hydrops fetalis (33%). 14/17 of diagnoses would have been made on antenatal findings alone while 3/17 additional diagnoses were made with findings only apparent on autopsy.

Conclusion: ES in pregnancy following the detection of structural anomalies on ultrasound is feasible and returns comparable diagnostic yields to ES combined with autopsy.

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P01.23C

From diagnostic yield to clinical impact: implementation of prenatal exome sequencing in routine car

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Introduction: Exome sequencing (ES) is an efficient tool to diagnose genetic disorders postnatally. Recent studies show that it may have a considerable diagnostic yield in fetuses with structural anomalies on ultrasound. We report on the clinical impact of the implementation of prenatal ES (pES) for ongoing pregnancies in routine care.

Material and Methods: We retrospectively analyzed the impact of pES on pregnancy outcome and pre- or perinatal management in the first 22, consecutively referred patients to our department for pES because of one or more structural anomalies on fetal ultrasound.

Results: In two cases, a diagnosis was made by chromosomal microarray analysis after ES counselling. The remaining 20 cases were divided in three groups; (1) pES to aid parental decision making (n = 12), (2) pES in the context of late pregnancy termination requests (n = 5) and (3) pES to guide prenatal or perinatal management (n = 3). pES had a clinical impact in 75% (9/12), 40% (2/5) and 100% (3/3) respectively, showing an overall clinical impact of pES of 70% (14/20).

Conclusion: We show that clinical implementation of pES is feasible and affects parental decision making or prenatal and perinatal management supporting further implementation of ES in the routine prenatal setting.

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P01.24D

Diagnosis of fetal structural abnormalities using whole exome sequencing: a single centre study

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Introduction: Fetal abnormality detected on ultrasound occurs in up to 5% of fetuses. In the absence of aneuploidy or cytogenetic causes, single gene disorders can be responsible; a recent review of 31 prenatal exome sequencing studies by Best et al., (2018) showed a diagnostic rate of 6.2% to 80%. We have undertaken exome sequencing at a single centre for cases of unexplained fetal anomaly in both ongoing and retrospective pregnancies.

Materials and Methods: Patients with a fetal abnormality detected on ultrasound were referred to clinical genetics at St George's Hospital, London. Fetal DNA was from CVS, amniotic fluid, fetal blood or tissue. Maternal cell exclusion was performed where relevant. DNA was enriched using the Agilent SureSelect CREv2 and sequenced using Illumina NextSeq 500. Variant analysis performed using Sapienita™. Gene panels and variant filter settings including Exomiser prioritisation were preconfigured to expedite analysis.

Results: In the first year (2018), 42 families were referred for exome sequencing due to fetal anomaly. A diagnosis was made in 15 cases (36%); 9/17 singletons (*BICD2, FGFR2, LZTR1, NIPBL, PTPN11, RAF1, RMRP, SLC26A2, TSC1*), 1/1 duo (*UBE2A*), 4/23 trios (*CHD7, PIEZO1, POMGNT1, SLC6A9, TUBA1A*) and 0/1 quad. Updated figures will be presented.

Conclusions: The diagnostic rate for this selected group is consistent with the literature and is higher than recent unselected cohort studies (Petrovski et al 2019, Lord et al 2019). In several cases the molecular diagnosis was not the primary suspected clinical diagnosis emphasising the continued importance of expanding and publishing prenatal genotype-phenotype associations.

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P01.25A

Chorionic villi sampling and QF-PCR + SNP-array routinely offered in early pregnancy losses

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Background: Genetic investigation is not routinely offered in early pregnancy loss due to its high failure and maternal cell contamination rates.

Objective: To identify the genetic causes of early pregnancy losses, with the use of QF-PCR and SNP-array in chorionic villi samples obtained previously to evacuation.

Methods: Women with a pregnancy loss before 13 weeks were offered transcervical chorionic villi sampling prior to surgical or medical uterine evacuation. During a 3-year study period (September 2015-October 2018) 584 women consented. A first round of QF-PCR including chromosomes 21, 18, 13, X, Y and a second round with chromosomes 15, 16, and 22 were assessed. Concurrent karyotyping was also performed and SNP-array when the chromosomes were normal.

Results: Fifty-five (9%) samples were excluded after microscopic inspection because only maternal decidua could be retrieved. Among the 529 samples suitable for analysis, a chromosomal anomaly was found in 159 (30%) cases at the first QF-PCR round, and 128 (24%) at the second round. The karyotype revealed 66 (12%) anomalies undetected by QF-PCR. Among the 176 remaining early losses with a normal karyotype, SNP-array was performed in 90 cases with sufficient DNA ad 6 (2.4%) submicroscopic anomalies were found.

Conclusions: QF-PCR and SNP-array in chorionic villi samples are able to provide a result in 91% of early pregnancy losses. Two rounds of QF-PCR detected a chromosomal anomaly in 54% of the cases. If karyotyping is replaced by SNP-array in QF-PCR normal cases, further 12% chromosomal anomalies, 2.4% submicroscopic anomalies and molar pregnancies will be revealed.

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P01.26B
NGS based multigene panel analysis in cases of fetal brain malformations

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Introduction: Identifying the genetic cause of fetal brain malformations is a diagnostic challenge due to the genetic heterogeneity. We present here the results of multigene panel analysis (MGPA) of 65 fetuses with congenital brain malformations.

Materials and Methods: From January to December 2018, 65 prenatal cases with fetal brain malformations (10 with minor extracerebral anomalies) were investigated by targeted Next Generation Sequencing of the coding regions of 262 genes. Genes were selected from the developmental brain disorders database (DBDB) and results reported within two weeks. Familial co-segregation analysis for variant pathogenicity assessment was carried out, when necessary. Karyotypes and array-CGH results of fetuses were normal.

Results: Pathogenic variants were identified in 10 (15%) out of the 65 cases. Mutations were found in a total of 8 genes: TUBA1A and ASPM (2 cases respectively) as well as in GPSM2, FKRP, KAT6B, OFD1, TSEN54 and TUBB (1 case each). In addition variants of unclear significance were detected in 4 cases (6%). No mutation was identified in a total of 51 fetuses (79%). Our findings show a strong correlation between the detection rate of causative mutations and complexity of the fetal phenotype. Additionally, Trio-based whole exome sequencing should be considered in cases with negative MGPA results to further improve the detection rate.

Conclusions: MGPA facilitates the identification of genetic causes with a good diagnostic yield and should be used for routine molecular genetic diagnosis of fetal brain malformations.

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P01.27C
A case of fetal hydrops and *FOXP3* - another argument for using exomes in pregnancy for isolated hydrops

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Introduction: Whole Exome Sequencing (WES) is currently entering prenatal genetic diagnosis for unsolved fetal malformations. Diagnostic rates vary significantly, but with low diagnostic rates in isolated increased nuchal translucency (NT) or cystic hygroma [Best et al 2018].

Materials and Methods: G5P2 with severe hydrops in 2 male fetuses (missed abortion week 19 and 20) and healthy children. Both hydrope fetuses presented with normal first trimester scans. Cytomegalovirus (CMV) was the presumed cause for the first case of hydrops, as the mother seroconverted just after the pregnancy. However, PCR for CMV was negative in tissue from the aborted fetuses. Array-CGH was carried out on DNA obtained from amniotic fluid from both pregnancies, but leaving both cases unresolved. WES was performed as trio on DNA extracted from amniotic fluid from the second pregnancy, and both parents. Results were validated by Sanger sequencing.

Results: A novel variant in *FOXP3* was identified in hemizygous state in the second pregnancy, inherited from the mother, and subsequently confirmed in cell culture from the first pregnancy. This variant is currently under further validation.

Conclusions: Germline *FOXP3* mutations cause immune dysregulation, polyendocrinopathy and enteropathy, X-linked (IPEX) syndrome. This syndrome is well characterized in patients postnatally. Prenatally, mutations have been reported to cause the loss of male fetuses as a result of fetal hydrops [Reichert et al. 2015]. This case adds to the body of knowledge suggesting that WES can improve our understanding of prenatal presentation of not only fetal malformations but also isolated hydrops.

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P01.28D
Absence of AGG interruptions is a risk factor for a full mutation expansion among ethnically diverse FMR1 premutation carriers

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Introduction: Genetic counseling given to FMR1 premutation carriers is currently based upon the number of CGG repeats. However, recent studies revealed that AGG interruptions may decrease the risk of intergenerational expansion.

Materials and Methods: All FMR1 premutation carriers who underwent chorionic villus sampling (CVS) or amniocentesis (AC) at Sheba Medical Center during the period of 2011-2017 were included in this study. To increase the accuracy of our results, we combined results from Israel with those from the New York State Institute for Basic Research in Developmental Disabilities (IBR). FMR1 PCR and Asuragen Kit were used to determine the number of CGG repeats and AGG interruptions in all women and fetuses.

Results: The combined data included 1471 transmissions of maternal premutation alleles: 369 (25.1%) stable and 1,102 (74.9%) unstable transmissions. Full mutation expansions were identified in 20.6% (303/1471) of transmissions. A total of 97.4% (388/397) of transmissions from alleles with no AGGs were unstable, compared to 79.6% (513/644) in alleles with 1 AGG and 46.7% (201/430) in alleles with 2 or more AGGs. 40% (159/397) of alleles with no AGGs expanded to a full mutation, compared to 20.2% (130/644) for alleles with 1 AGG and only 3.2% (14/430) in alleles with 2 AGGs or more.

Conclusions: Based on this ethnically diverse data we recommend that the risk estimates for a full mutation expansion for FMR1 premutation carriers will include the number of AGG interruptions as well as CGG repeat size. Study funding/competing interest(s) - Azrieli foundation, Canada-Israel

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P01.29A
Targeted next-generation sequencing and CNV analysis identifies two novel mutations in the FRAS1 gene

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Introduction: We report on a case of a nonconsanguineous couple with one healthy child and two spontaneous abortions (one at 22 and one at 36 weeks of gestation). Test results of the couple's chromosome analysis were normal, and there was no evidence of genetically relevant diseases in their families. Frozen skin cells of the first abort had an insufficient quality to perform gene panel analysis.

Materials and Methods: Clinical exome sequencing (CES) was performed on the parents' DNA followed by screening for carrier status in 34 relevant genes for intrauterine fetal death.

Results: In the maternal DNA sample, we identified a heterozygous novel mutation c.2423-1G>T in the FRAS1 gene affecting a highly conserved donor splice site and leading most probably to aberrant splicing. Various splice site mutations in the FRAS1 have been described before. In the paternal DNA sample, copy number variation (CNV) analysis based on next-generation sequencing data showed a novel heterozygous deletion of the last three exons of the FRAS1 gene. Junction fragment PCR confirmed this deletion and sequencing of the PCR products revealed the breakpoints (c.11092+658_*12039+6368del). Although the material of the first abort had poor quality, Sanger sequencing and junction fragment PCR on frozen skin cells were able to identify these two novel variants as compound heterozygous.

Conclusion: Therefore, clinical exome sequencing in combination with CNV analysis on a couple's DNA is an appropriate method to detect possible causes of multiple stillbirths, especially when no fetal DNA is available.

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P01.30B
Identification of compound heterozygous *FTO*-mutations in a severe malformation syndrome

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We report on a non-consanguineous couple who presented in the 19th week of their 6th pregnancy with ultrasound abnormalities of the fetus (broad neck with increased nuchal translucency, multicystic dysplasia of the kidney, short femur, fetal clubfoot). The couple has got just one healthy child. Two spontaneous abortions occurred and one pregnancy with trisomy 21 was terminated. Another child also exhibited a broad neck and additional omphalocele early in pregnancy. Hydrocephaly was first visible in the 32nd week. After delivery in the 35th week, she required artificial respiration. MRI showed a complex brain

malformation. That child deceased at the age of one month. Chromosome analysis and microarray were normal in both malformed children. After termination of pregnancy we performed a clinical trio-exome (Agilent SureSelect Inherited Disease Panel). We identified two loss-of-function mutations in compound heterozygosity in the *FTO* gene not yet described (ACMG class 4). The deceased sibling carried both mutations and the healthy sister none. Homozygous *FTO* loss-of-function mutations have been associated with multiple malformations including short broad neck, different brain malformations, severe developmental delay, failure to thrive and high risk of death in early childhood (Boissel 2009, Daoud 2016). In summary, the *FTO*-mutations are very likely to be causative for the disease because of the type of mutation, the phenotype and the segregation data. Nevertheless, prenatal molecular analysis should be accompanied by a detailed ultrasound work-up. Moreover, the couple has to be aware that the absence of the *FTO*-mutations does not completely exclude the disease.

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P01.31C

Early prediction of preeclampsia (PE) severity in pregnant women with preexisting type 1 diabetes mellitus (T1DM): new implements for genetic markers

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Background: Severe PE strongly correlates with persistent disability, long-term cardiovascular complications in mothers and prematurity, IUGR or BPD in neonates. The study objective was to establish reliable clinical, genetic markers associated with PE severity in T1DM women.

Materials and Methods: Group 1 included patients with mild (n=15), moderate (n=11), severe (n=4) PE; Group 2 - 30 patients, in whom pregnancy was not complicated with PE. Polymorphisms in *eNOS* (4b/4a, G894T), *ACE* (I/D), AT2R1 (A1166C), MGP (Thr83Ala, T138C) and PON1 (C108T) genes were detected using PCR-RFLP. Data was analyzed with SPSS17.0 using paired sample t-test, Pearson's chi-squared test with 95%CI calculation for OR. Binary logistic regression was used to generate statistical models and evaluate their prognostic value. ROC curve analysis was performed in order to establish the influence of polymorphic variants of genes on PE severity.

Results: Main contributors to moderate/severe PE development: pre-existing vascular complications (nephropathy [$\chi2$=10,05, p=0,004, OR=11,23 95%CI 2,13-59,26], lower extremities angiopathy [$\chi2$=7,39, p=0,007, OR=16,00 95%CI 1,86-137,61], retinopathy [$\chi2$=4,27, p=0,039, OR=6,50 95%CI 1,25-33,91]) and their combinations (nephropathy + retinopathy; nephropathy + lower extremities angiopathy) and ACE DD-genotype. In PON1_108CT carriers clinical parameters exerted modifying effect: when combined with nephropathy, or T1DM duration < 8,5 years, or BMI < 23,8 kg/m^2, mild PE develops; moderate/severe PE develops when diabetes duration is > 13,5 years or BMI is > 25,17 kg/m^2. Combination ACE_II/PON1_CC was protective against PE development, while ACE_ID/PON1_CT is associated with mild PE. **Conclusion:** Genetic markers are helpful in identification of women at high risk for severe PE.

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P01.32D

Case of 8p and 18p genetic imbalance in a subfertile female patient without pronounced physical and mental abnormalities

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Introduction: We report an unusual case of unbalanced karyotype female carrier with no physical and mental abnormalities and her reproductive history.

Materials and Methods: Conventional karyotyping (GTG and QFH/AcD-banding techniques) was performed on the chorionic villi from aborted material and on PHA-stimulated lymphocytes of the patient, her husband and patient's parents. FISH and aCGH (CGXv1.1 8x60K,PerkinElmer) were performed on the patient's PHA-stimulated lymphocytes and whole blood sample, respectively. Standard IVF was performed with an embryo biopsy on day 3 (6-8-cell stage) for PGT-SR by FISH.

Results: The patient - 28-years old women - sought genetic counseling after loss of two naturally conceived pregnancies. The first miscarriage was not karyotyped. The second had karyotype 45,XX,der(8)t(8;18)(p23;p11.3),-18mat. The patient appeared to have the same karyotype: 45,XX,der(8)t(8;18)(p23;p11.3),-18dn. aCGH revealed terminal deletions combined with microduplication: del(8)(p23.1p23.3) (6.718Mb), dup(8)(p22p22) (4.937Mb), del

(18)(p11.31p11.32) (3.693Mb), dup(18)(q23q23) (0.060Mb). The patient's husband and parents had normal karyotypes. The patient had only slight dysmorphic face features and no pronounced physical and mental abnormalities. Subsequently, the patient underwent four IVF cycles with a total of 25 oocytes obtained. Only 13 of them reached MII (52% versus expected 75-90%). Only one out of 10 embryos was cytogenetically balanced. The embryo was transferred, but the pregnancy was not registered.

Conclusions: The present case demonstrates a genotype-phenotype disparity in the carrier of unbalanced chromosomal rearrangement. Considering an unpredictable effect of the genetic imbalance on the offspring phenotype, a personalized approach to the patient's genetic counseling is required in order to achieve pregnancy with cytogenetically balanced embryo.

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P01.33A

Trans-ethnic meta-analysis meta-analysis of gestational diabetes reveals shared genetic background with type 2 diabetes

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Gestational diabetes mellitus (GDM), defined as glucose intolerance first recognized in pregnancy, has important implications for both mother and child. Offspring of mothers with GDM have an increased risk of birth complications associated with higher risk for developing metabolic syndrome, type 2 diabetes (T2D) and cardiovascular disease in later life. To date, the only genome-wide association study (GWAS) for GDM, in 1,399 affected Korean women, revealed associations at two loci: *MNTR1B* and *CDKAL1*. We conducted trans-ethnic meta-analysis of 21 GWAS in 5,374 cases and 346,506 controls of diverse ancestry (62.0% European, 16.5% East Asian, 3.2% Mexican-American and 18.3% Afro-Caribbean), each imputed up to reference panels from the 1000 Genomes Project or Haplotype Reference Consortium. The trans-ethnic meta-analysis, performed with MR-MEGA to allow for heterogeneity in allelic effects between ancestries, included 13,980,490 variants, after excluding those with minor allele count <5 and imputation quality <0.4 in each GWAS. We replicated both known GDM associations at genome-wide significance ($P<5x10^{-8}$): *MTNR1B* (rs10830963, $P=2.3\times10^{-49}$) and *CDKAL1* (rs9348441, $P=9.7\times10^{-15}$). We also identified three additional novel loci: *TCF7L2* (rs7903146, $P=1\times10^{-14}$), *CDKN2A/B* (rs10811660, $P=1.9\times10^{-9}$) and *LOC105369513* (rs143421658, $P=4.1\times10^{-8}$). Allelic effects of GDM association signals were mostly homogeneous across ethnic groups. The exception was at the *CDKAL1* locus ($P_{het}=3.68\times10^{-9}$), where the signal was driven by East Asian ancestry GWAS ($OR_{East\text{-}asian}=1.67(1.46\text{-}1.89)$; $OR_{Europeans}=1.06(1.01\text{-}1.12)$). Four of the loci (*MTNR1B*, *CDKAL1*, *TCF7L2*, and *CDKN2A/B*) have also been robustly associated with T2D, and lead variants are identical to those we have identified for GDM, supporting a shared underlying genetic contribution to both diseases.

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P01.34B

Genome-wide analysis of losses and gains a better understanding of idiopathic male infertility

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Introduction: Diagnosting workup of infertile man, which include screening for chromosomal aberrations and Y chromosomal azoospermia factor deletions, have not been

changed for years and still the etiology of most of the infertile cases remains unknown. Aiming to achieve progress in elucidating genetic causes of male infertility and to examine suitability of novel genetic testing methods in clinical routine of infertile man, we investigated man with unexplained infertility with Array Comparative Genomic Hybridisation method.

Materials and Methods: We enrolled 90 patients with severe male factor infertility, defined as idiopathic azoospermia or oligoasthenoteratozoospermia, and analyzed DNA using SurePrint G3 Unrestricted CGH 4x180K microarrays. We have selected candidate Copy Number Variants (CNVs) according 2 criterias: 1st criteria was that identified CNVs are not present in currently available databases of genomic variants, and 2nd criteria was that identified CNVs are not present in our control data set of men with proven fertility.

Results: We identified 5 CNVs which included genes previously related to male infertility (CLCA4, USF1, FCER1G, FNTA, HOTAIR). We have also identified 7 CNVs not presented in currently available databases of genomic variants or in our control data set. The identified CNVs spanning genes with noticed expression in testis, however, their possible functional impact on infertility are currently not known.

Conclusion: We provide a several candidate CNVs, possibly implicated in male infertility. Taken together with previous research, these findings are one more step forward to implementation of new testing methods into routine clinical practice of infertile man.

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P01.35C

Detection of *de novo* copy-number variations from exome sequencing of 108 infertile patient-parents trios

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Introduction: Approximately 7% of all men are infertile and genetics is known to play an important role in the most severe forms of infertility such as azoospermia and extreme oligozoospermia. However, most patients remain undiagnosed. In this study, we explored the role of *de novo* copy-number variations (CNVs) in whole-exome sequencing (WES) data from a unique cohort of 108 infertile men and their parents.

Material and Methods: Three different software, CoNIFER, XHMM and an optimized GATK4 pipeline were used to detect rare *de novo* CNVs. XHMM and GATK4 reduced the inherent noise in the exome coverage data best, resulting in the most accurate CNVs detection. Additionally, the GATK4 pipeline allowed to integrate allele frequency analysis, confirming the loss of heterozygosity (LOH) in deletions.

Results: Application of these tools revealed two rare *de novo* deletions in two different patients. One deletion occurred on chromosome 11 and partially overlapped a deletion previously reported in an infertile man. The second, affected *NXT2* on chromosome X, a gene evolutionary conserved and highly expressed in testis. Both CNVs were validated by microarrays and/or Q-PCR.

Conclusions: These first data indicates that *de novo* CNVs may play an important role in severe male infertility. However, replication and functional studies are required to further validate the impact of our findings. By further improving CNV detection from WES data we may be also able to identify additional *de novo* CNVs that are currently missed, as well as maternally inherited CNVs that may play a role in male infertility.

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P01.36D

Identification of a Novel Genetic Cause of Familial Nonobstructive Azospermia

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Infertility is a global reproductive health problem, and in males it is usually because of the reduced number or the complete absence of sperm cells in semen (oligozoospermia and azoospermia, respectively). Nonobstructive azoospermia (NOA) is the most severe form of male infertility affecting ~0.6% of men from the general population and ~10% of infertile men. Its etiology remains largely unknown. To identify the genetic cause of NOA in four affected members from a consanguineous family, we performed whole-exome sequencing (WES). WES revealed a homozygous c.1166C>T (p.Pro389Leu) variation in the *M1AP* gene. The segregation of the *M1AP* variant with NOA in this family was confirmed by Sanger sequencing. The 3D structure of the mutant protein was predicted computationally. Sequence- and structure-based *in silico* studies and subsequent preliminary gene expression studies imply that the *M1AP* variant has severe implications for protein structure and function. Further functional studies to corroborate our findings are ongoing. Overall, *M1AP* is a novel candidate gene for male infertility and, to the best of our knowledge, this is the first report identifying *M1AP* as a cause for human familial NOA. And our pedigree analysis suggests an autosomal recessive mode of inheritance for NOA due to *M1AP* in the present family.

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P01.39C

Genetic analysis of 24 candidate genes validates TEX14, TEX11, NR5A1 and DMRT1 as clinically relevant for non-obstructive azoospermia

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Male infertility is a clinically and genetically highly heterogeneous disease, mostly characterised by spermatogenetic failure, clinically noted as oligo- or azoospermia. The most common form of the latter phenotype is non-obstructive azoospermia (NOA), which can be caused by various genetic defects such as chromosomal aberrations, Y-chromosomal AZF microdeletions or monogenic defects. In this study, we systematically analysed 24 genes reported to be associated with NOA in the OMIM database. Variants in these genes were detected in whole exome sequencing data from 484 well-phenotyped infertile patients (azoospermia, N=94; cryptozoospermia, N=52; mixed atrophy, N=93; Sertoli-Cell-Only syndrome, N=179; meiotic arrest, N=43; other arrest, N=23). Other clinical causes for their infertility such as previous chemo-/radiotherapy, as well as karyotype aberrations and AZF deletions had been excluded. Exclusively novel or rare coding variants were assessed concerning their potential pathogenicity. Out of the 24 genes listed in OMIM, *TEX14, TEX11, NR5A1* and *DMRT1* were validated as clinically relevant genes causing NOA with strong evidence. Specifically, we identified eight patients carrying *TEX11* variants. Ten patients demonstrated compound heterozygous or homozygous *TEX14* variants. Five patients carried *NR5A1* and five others *DMRT1* variants. This is the first report of concise exome sequencing in a large group of infertile males. Our results clearly demonstrate that the *TEX14, TEX11, NR5A1* and *DMRT1* genes have reached a sufficient level of evidence to be prioritised for clinical analyses. 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

Maternal copy number variations in the *DMD* gene as incidental findings in non-invasive prenatal testing

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Noninvasive prenatal testing (NIPT) using shallow whole-genome sequencing also reveals maternal copy number variations (CNV's). Some of those variants are clinically actionable or could be harmful for the fetus. CNV's in the *DMD* gene, potentially causing dystrophinopathies, are amongst the most commonly observed maternal CNV's. We analyzed the data of maternal CNV's detected in the *DMD* gene by NIPT. Out of 26.123 NIPT analyses, 16 maternal CNV's in the *DMD* gene were detected (1/1.632 pregnant women). Variant classification regarding pathogenicity and phenotypic severity was based on public databases, segregation analysis in the family and a prediction of the effect on the reading frame. Ten CNV's were classified as pathogenic, 4 as benign whereas 2 remained unclassified. We present our experience with the detection of maternal CNV's in the *DMD* gene and propose a scheme for the interpretation and the returning of these CNV's detected by NIPT. We show that genome-wide NIPT leverages CNV screening in the general population of pregnant women and that interrogating the maternal CNV landscape can improve overall pregnancy management.

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P01.41A

Validation of SNP-based noninvasive prenatal screening test to detect maternal X chromosome abnormalities

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Introduction: Maternal chromosome abnormalities (CA) are known incidental findings with non-invasive prenatal screening (NIPS), contributing to false positives with quantitative methodologies. SNP-based NIPS can distinguish the maternal and fetal SNP allele distributions. This study validates the performance of SNP-based NIPS to detect maternal CA on chromosome X.

Methods: Plasma samples from singleton pregnancies were obtained (April-December 2018) and analyzed by SNP-based NIPS. Samples were included in the analysis if the algorithm could not return a result (below threshold for reporting) for suspected CA. These samples were stratified into suspected maternal (group A, n=107) and fetal origin (group B, n=110). Associated maternal buffy coats were blinded and sent to CHOP for cytogenetic analysis using the Global Screening Array (GSA, Illumina).

Results: Of 107 group A samples, 101 were confirmed by GSA to have maternal X CA (PPV: 94.4%; 97.5% confidence interval (CI), 88.2-100%); in the remaining 6 samples, 3 were discordant (GSA unable to detect suspected maternal CA), and in the other 3 samples, maternal CA was suspected, but too ambiguous to call as present. In 67.3% (68/101) cases, NIPS vs. GSA interpretations matched; and 32.7% (33/101) showed similar but varied interpretations due to ambiguities arising from mosaicism and/or specific X abnormality. No maternal X CA were found in group B by GSA (NPV: 100%; 97.5% CI, 96.7-100%).

Conclusions: The study supports the reporting of maternal X CA suspected by SNP-based NIPS, which was confirmed in 94.4% of suspected cases. No maternal abnormalities were identified when a fetal abnormality was suspected.

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P01.42B

Genetic signals for use of hormone replacement therapy in post-menopausal women suggest potential drug targets and reflect changes in health practice

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Introduction: Hormone replacement therapy (HRT) is an effective treatment for vasomotor symptoms around

menopause, but in the UK HRT usage dropped following the publication of health risks in 2002.

Materials and Methods: To understand the biology of HRT use, we carried out genome-wide analyses of self-reported HRT phenotypes (ever taken, age started, age stopped and time taken) in up to 153,152 white European women aged 40-70 years from the UK Biobank.

Results: We identified 15 independent signals. A signal (AF=95%) in *TACR3*, the receptor for neurokinin B, was associated with raised odds of using HRT (1.18 per allele; $P=1\times10^{-26}$) and with vasomotor symptoms in a previous genome-wide analysis (Crandall et al 2017). Additionally, a putative signal (AF=0.1%) near *ALDH8A1* (gene involved in tryptophan catabolism) was associated with taking HRT for longer (0.5 SD per allele; $P=4\times10^{-8}$). Tryptophan is a precursor of serotonin, which has been implicated in hot flushes, as has neurokinin B.

Of the signals, 13/15 were associated with starting HRT younger at $P<5\times10^{-8}$ and 11 with earlier menopause. A genetically-predicted one year earlier menopause raised the odds of HRT use in women starting treatment before 2002 (OR=1.12, 95% CI=1.10,1.13) but not after 2002 (OR=1.02, 95% CI=0.95,1.00). In contrast, the signals near *TACR3* and *ALDH8A1* were not associated with menopause timing.

Conclusions: Genetic associations for HRT are largely driven by menopause timing and are affected by changes in health practice. We identified two genetic signals, which are not affected by health practice, near plausible candidate genes for vasomotor symptoms.

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P01.43C
Prenatal diagnostics: the utility of molecular karyotyping

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Introduction: the aim of this study is to highlight the use of molecular karyotyping in the context of prenatal diagnostic testing.

Materials and Methods: Molecular karyotype was applied on 4012 prenatal samples (CVS, amniotic fluids) as the first line diagnostic tool. Specimens have been analyzed using Agilent SurePrint G3 8x60K ISCA design and Cytogenomics software.

Results: women who underwent prenatal molecular karyotype were referred for ultrasound findings, increased NT, advanced maternal age, previous pregnancy with aneuploidy, positive biochemical or NIPT, anxiety etc.

Molecular karyotype shows an increased diagnostic yield compared to conventional karyotype varying from 1,4% to 3,4% depending on the referral reason with the highest added value in fetuses with abnormal U/S findings. Among all abnormal CGH samples with normal karyotype, 72,5% of clinically significant imbalances would have escaped detection from NIPT since they concern rare syndromes (25%), susceptibility to-loci (60%) and backbone abnormalities (15%) related to the referral reason.

Conclusion: Since more than 70% of the abnormal CGH cases would not be detected neither by conventional karyotype nor by NIPT, we suggest that every pregnant woman of any age should be offered the informed choice to undergo molecular karyotype prenatal testing.

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P01.44D
Prenatally diagnosed megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome

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Introduction: Megalencephaly-polymicrogyria-polydactyly-hydrocephalus (MPPH) syndrome is characterized by megalencephaly, polymicrogyria and a variant in one of three genes: *CCND2, AKT3* or *PIK3R2*. To the best of our knowledge, there is only one prenatally reported MPPH case. We present a new case of MPPH with a variant in *CCND2* that was diagnosed prenatally.

Case report: Chorionic biopsy was performed during the 1st trimester of the pregnancy due to increased NT value (3.39 cm) to a 29-years-old patient. The karyotype of the fetus was 46,XY. Ultrasonography and magnetic resonance imaging of the fetus showed extensive bilateral cortical dysplasia of the supratentorial cerebral parenchyma with a suspected focal acute lesion in the right parietotemporal

region. Dysgenesis of the corpus callosum as well as asymmetry of the hemispheres of cerebellum were also visualized. The radiological changes were nonspecific but indicated either intrauterine infection or genetic brain malformation. The pregnancy was ultimately terminated, and an autopsy revealed multiple congenital cerebral anomalies: abnormal cranial occlusion, abnormal additional clefts on right in the occipital and parasagital regions, dysgenesis of the corpus callosum, and a collapsed and hypoplastic septum pellucidum. There were no polydactyly and signs of inflammation in the placental and fetal tissues. To investigate the underlying etiology of this phenotype, trio exome sequencing (ES) analysis was done.

Result: ES revealed a pathogenic *de novo* missense variant: NM_001759.3(*CCND2*):c.839C>A p.(Thr280Asn) rs587777620, confirmed by Sanger sequencing.

Conclusions: MPPH syndrome should be considered when prenatally cerebral dysgenesis is visualized on US and/or MRI.

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P01.46B
Robust strategy for preimplantation genetic testing of myotonic dystrophy type 1 by bidirectional triplet-primed PCR combined with multi-microsatellite haplotyping following whole genome amplification

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Myotonic dystrophy type 1 (DM1) is caused by moderate to very large expansions of the *DMPK* CTG trinucleotide repeat. Disease transmission to offspring can be avoided through prenatal diagnosis or preimplantation genetic testing for monogenic disorders (PGT-M). We describe a robust PGT-M strategy that can be applied to virtually any couple at risk for DM1, including carriers of large expanded alleles with non-CTG interruptions at either the 5' or 3' end of the repeat. This strategy utilizes whole-genome amplification by multiple displacement amplification, followed by bidirectional triplet-primed PCR (TP-PCR) sizing of the *DMPK* CTG repeat, in parallel with single-tube genotyping and haplotype phasing of 12 closely linked and highly polymorphic microsatellite markers. Bidirectional TP-PCR and dodecaplex marker PCR assays were optimized and validated on whole-genome amplified single lymphoblasts isolated from DM1 reference cell lines, and tested on a simulated PGT-M case comprising a parent-offspring trio and three simulated embryos. *DMPK* TP-PCR reliably detects repeat expansions regardless of allele size, and employing TP-PCR in both directions ensures successful expansion detection even when non-CTG interruptions occur at either the 5' or 3' end of the expanded allele. Misdiagnoses and diagnostic ambiguity due to allele dropout or exogenous DNA contamination can be easily detected through the use of tightly linked microsatellite markers, minimizing the exclusion of potentially unaffected embryos for uterine transfer. The highly polymorphic multi-marker panel also maximizes the likelihood of marker informativeness in at-risk couples, thus minimizing the need for couple-specific assay customization.

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P01.47C
Nicotine and resveratrol alter sox2 and sox4 genes expression levels in human amniotic cell culture

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Introduction: The aim of this research is to investigate the effects of nicotine on expression levels of SOX2 and SOX4 master genes in human amniotic cell cultures to indicate the risks of smoking in pregnancy. The effects of resveratrol on expression levels of these genes in human amniotic cell cultures, which are treated by nicotine were also examined in this study.

Materials and Methods: Twenty patients were included in our study and for each patient; control, nicotine treated and nicotine + resveratrol treated cell culture groups are formed. The expression levels of SOX2 and SOX4 genes are examined in each group by using real time RT-PCR.

Results: According to the results of our study, change in expression levels of SOX2 and SOX4 genes in nicotine treated group were found to be statistically significant. Also, when groups treated with nicotine and nicotine + resveratrol were compared the difference was found to be statistically significant.

Conclusion: In conclusion, nicotine increased the expression levels of SOX2 and SOX4 genes by 60% in human amniotic cell cultures and resveratrol was found to be an important antioxidant that reduces the increased expression levels of SOX2 and SOX4 genes caused by nicotine treatment.

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P01.48D

A placental trisomy 2 detected by NIPT evolved in a fetal small Supernumerary Marker Chromosome (sSMC)

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Objective: Here we report a prenatally detected mosaicism of a small supernumerary marker chromosome (sSMC) derived from chromosome 2. The 38-year-old woman underwent amniocentesis because of a high risk of trisomy 2 revealed by the Non-Invasive Prenatal Test (NIPT).

Methods and results: A genome-wide NIPT detected a trisomy of chromosome 2. The amniocentesis was performed to verify the NIPT result. Array comparative genomic hybridization (aCGH) from uncultured amniocytes revealed a duplication of 14,83 Mb on chromosome 2q11.1q13. Interphase fluorescence in situ hybridization (FISH) revealed three signals of centromere 2 in 30% of the cells. GTG-banded metaphases confirmed the abnormal karyotype (47,XX,+mar[21]/46,XX[19]), indicating 52% mosaicism of the cell line with the sSMC. The ultrasound examination did not reveal abnormalities. The pregnancy has been terminated. Cytogenetic analyses (FISH, aCGH and conventional karyotype) on fetal skin biopsies were performed and confirmed the genomic gain of the centromeric region of chromosome 2. In the placenta, three cell lines were detected: a normal cell line, a cell line with trisomy 2 and a third one with only the sSCM derived from chromosome 2. Conclusion: Whole-genome NIPT allows not only the identification of common fetal trisomies (13, 18, 21) but also diagnosis of rare chromosomal abnormalities. Especially in such cases, it is extremely important to perform not only NIPT verification on a sample of material other than trophoblast, but also to apply appropriate research methods. Such conduct allows detailed analysis of the detected aberration, thus appropriate clinical validity.

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P01.49A

Non-Invasive Prenatal Testing pitfalls: exceptional report of multiple discrepancies between noninvasive screening for fetal trisomy 21, karyotype, array CGH and fetal ultrasound

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Introduction: Pitfalls of noninvasive prenatal test (NIPT) for fetal trisomy 21 on circulating cell-free DNA are currently well known and are linked to confined placental mosaicism, maternal or fetal mosaicism, maternal neoplasia, evanescent twin, or low fetal DNA fraction. Unbalanced chromosomal rearrangements may also interfere with NIPT. We report here a rare anomaly identified through NIPT.

Case: A NIPT was performed on a 39-year-old woman with abnormal first trimester serum markers. This NIPT (*Clarigo, Multiplicom Agilent*) did not show any trisomy 21 but was evocative of trisomy 18. An amniocentesis was performed at the 22th week of gestation (WG) and FISH analyses (*Aneucyte, Cytocell)* on native amniotic fluid were compatible with a trisomy 18 in a female fetus. Unexpectedly, concomitant ultrasound control didn't detect any morphological abnormality on a male fetus. Then, fetal karyotype and array-CGH confirmed the female chromosomal sex and revealed a very partial trisomy 18, linked to a small supernumerary marker chromosome, in mosaic, which was derivative from a chromosome 18. In addition, *SRY* locus was found on the short arm of one X chromosome. Genetic counseling being less alarming in this context than with a complete trisomy 18, this patient pursued her pregnancy and delivered a boy at 36 WG with normal neonatal examination. A regular monitoring of his psychomotor development is under way.

Discussion: This observation underlines the necessary precautions while interpreting NIPT and the necessity of a

strong cytogenetic confirmation. This case also illustrates that incidental findings are to be expected with NIPT.

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P01.50B
Pilot study of locally performed noninvasive prenatal testing NIPT in Bulgaria

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Introduction: With the introduction of noninvasive prenatal testing (NIPT), the prenatal screening for chromosomal aneuploidies has been expanded beyond ultrasound examination and combined first-trimester screening (cFTS). NIPT using cell-free DNA (cfDNA) circulating in maternal blood presents as an early, accurate, and safe prenatal testing. Since August 2018 NIPT for chromosomes 21, 18, 13 and sex determination was introduced in-situ in the University Hospital of Obstetrics and Gynecology "Maichin dom", Sofia. The analysis was discussed with pregnant women after performing cFTS.

Materials and Methods: A total of 134 pregnant women were screened for chromosomal aneuploidies by NIPT using ion semiconductor sequencing platform (Ion Proton™, Thermo Fisher Scientific) and IONA® test (Premaitha Health plc, Manchester, UK). The patients were at a mean age of 34.5 years. Plasma samples were collected between 10+5 and 24+5 g.w.

Results: Due to an intermediate risk (1/100 to 1/1000) NIPT was performed on 68 (47.76%) pregnant women. Others were tested because of maternal anxiety or advanced age. An average of 10.6% (from 3 to 24%) fetal fraction (FF) was achieved. One report was unsuccessful due to a low fetal fraction in patient with BMI=43.03. We obtained 132 "low risk" reports and one "high risk" - for trisomy 21, confirmed by amniocentesis followed by QF-PCR.

Conclusions: This is the first introduction of locally performed NIPT in Bulgaria. With the accumulation of more samples and data a possible correlation between cFTS results and NIPT; FF or/and gestational week and NIPT results could be established.

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P01.51C
Unexpected finding of uniparental disomy mosaicism in term placentas: Is it a common feature in trisomic placentas?

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Objective: Non-invasive prenatal testing (NIPT) detects placental chromosome aberrations. When amniocentesis reveals a normal karyotype, confined placental mosaicism (CPM) may be assumed. In order to confirm this, placental cytogenetic studies were performed.

Method: NIPT was conducted in the course of the DutchTRIDENT study. Placentas of 10 cases with NIPT results indicating an autosomal trisomy and showing a normal (N = 9) or low mosaic karyotype (N = 1) in amniotic fluid (AF) were investigated. The cytotrophoblast as well as the mesenchymal core of two to four placental chorionicvilli biopsies were studied with single nucleotide polymorphism (SNP) array. Clinical outcome data were collected.

Results: In 10/10 cases, CPM was proven. In 3/10 cases trisomy/uniparental disomy (UPD)/biparental disomy (BPD) mosaicism was discovered. In 2/3 cases, all three cell lines were present in the placenta, whereas BPD was found in AF. In 1/3 cases trisomy 22/UPD22 was present in AF while trisomy 22/BPD22 mosaicism was found in the placenta. Five of 10 pregnancies were affected with pre-eclampsia, low birth weight, preterm delivery, and/or congenital malformations.

Conclusion: The presence of trisomy/UPD/BPD mosaicism in 3/10 cases that we investigated proves that trisomic zygote rescue may involve multiple rescue events during early embryogenesis. UPD mosaicism, when present in crucial fetal tissues, may explain the abnormal phenotype in undiagnosed cases.

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P01.53A
Non-Invasive Prenatal Testing: Laboratory Clinical Experience: 20000 Clinical Samples

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Introduction: Whole-genome sequencing (WGS) of maternal plasma cell-free DNA (cfDNA) can potentially evaluate all 24 chromosomes to identify abnormalities of the placenta, fetus, or pregnant woman. The objective of this study is to give a complete and robust clinical picture of the current performance of NIPT for trisomy 13, 18, and 21 and sex chromosomes aneuploidies as well as for the other chromosomes.

Materials and Methods: All data were generated in our AMES accredited laboratory from January 2017 to January 2019 in 20000 samples. The pipeline included automated library preparation (VeriSeq NIPT Microlab STAR, Illumina) and WGS sequencing on a Next550 (Illumina). VeriSeq NIPT Assay Software (www.illumina.com/NIPTsoftware) was used for data analysis of aneuploidy status of 13, 18, 21, X and Y chromosomes and fetal fraction of cffDNA. An in house algorithm was optimized to analyze other aneuploidies and subchromosomal aberrations.

Results: The main results were showed in Table 1. We also reported 19 rare autosomal trisomies (RATs) and 6 structural abnormalities identified in 10500 samples, mainly trisomy 15 (n = 5), followed by trisomy 8 (n = 4) and trisomies 16 and 22 (n=2).

Conclusions: The performance characteristics were established in samples in which we analysed all 24 chromosomes with a minimum fetal fraction of 4%, and has been confirmed by our extensive clinical experience in the same clinical population.

Table 1: Clinical performance based on clinical Experience

Overall performance n=20000	TP	FP	FN	Sensitivity (% (95% Cl))	Specificity (% (95% Cl))
T21	**151**	**1**	**0**	**100**	**100**
				(97.52,100.0)	(99.97, 100.0)
T18	**44**	**6**	**0**	**100**	**99.97**
				(91.97, 100.0)	(99.93, 100.0)
T13	**24**	**4**	**0**	**100**	**99.98**
				(86.2,100.0)	(99.95, 100.0)
Sex chromosome aneuploidies	**80**	**14**	**0**	**100**	**99.93**
				(95.42,100.0)	(99.88, 99.96)
All	**299**	**25**	**0**	**100**	**99.87**
				(98.73,100.0)	(99.81,99.91)
Multiple gestation n =441	**TP**	**FP**	**FN**	**Sensitivity (% (95% Cl))**	**Specificity (% (95% Cl))**
T21	**5**	**0**	**0**	**100**	**100**
				(56.55,100.0)	(99.13, 100.0)
T13	**0**	**1**	**0**		**99.77**
					(99.13, 100.0)
ART pregnancies n=984	**TP**	**FP**	**FN**	**Sensitivity (% (95% Cl))**	**Specificity (% (95% Cl))**
T21	**2**	**0**	**0**	**100**	**100**
				(34.24,100.0)	(99.61, 100.0)
T18	**2**	**0**	**0**	**100**	**100**
				(34.24,100.0)	(99.61, 100.0)
T13	**0**	**1**	**0**		**99.77**
					(99.13, 100.0)
Sex chromosome aneuploidies	**7**	**3**	**0**	**100**	**99.69**
				(64.57,100.0)	(99.10, 99.69)

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P01.54B
High total DNA contributes to low fetal fraction in NIPT and tends to resolve on redraw

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Introduction: Low fetal fraction (FF) is a common cause of test failures in NIPT and is correlated with maternal weight, gestational age, and aneuploidy[1,2]. We show that low FF can also be associated with high DNA concentration (DC) which frequently reverts toward average after redraw.

Materials and Methods: Sample DC was estimated by comparing to a reference sequence added before amplification. 99,936 eligible samples were collected between 2012 and 2014. Additionally, 1,374 received a redraw after test failure and changes in DC and FF between the two draws were observed.

Results: Samples with low DC (below 10th percentile) have average FF 12.4% and samples with high DC (above

90th percentile) have average FF 8.6%. The average FF is 10.1%. Samples with test failure were twice as likely to have high DC. After redraw, cases with high initial DC had 38 times larger median reduction in DC compared to overall. The 10% of cases with greatest reduction in DC had median FF increase 1.2%, compared to FF increase 0.4% for cases without significant change in DC. Change in DC was a significant parameter ($p<10-5$) in a logistic regression model for redraw success.

Conclusions: Samples with high DC are overrepresented in test failures and have lower average FF. High DC tends to reduce after redraw, producing larger FF increase. This supports the option for repeat NIPT after a failure due to low FF.

[1] Pergament et al. Obstet Gynecol. 2014 Aug;124(2 Pt 1):210-8.

[2] McKanna et al. Ultrasound Obstet Gynecol. 2019 Jan;53(1):73-79.

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P01.55C
Microarray findings in pregnancies with oligohydramnios - a retrospective cohort study and literature review

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Introduction: This study was performed following a statement in national position paper defining the advised management of pregnancies with abnormal amniotic fluid volume. The proposed statement recommended to refer all pregnancies with oligohydramnios to genetic counseling, except for proven rupture of membranes, evidence of placental insufficiency or oligohydramnios diagnosed after 37 weeks of gestational age. Thus, the objective of this study was to explore the risk for abnormal chromosomal microarray analysis (CMA) findings in pregnancies with oligohydramnios.

Methods: Data from all CMA analyses performed due to oligohydramnios between January 2013 and September 2017 were retrospectively obtained from the Ministry of Health database. In addition, a search was conducted through the Pubmed database from inception to February 2018 for English articles exploring the issue.

Results: Fifty CMA analyses were performed due to oligohydramnios. Of these, in 21 tests it constituted an isolated anomaly, 13 cases were associated with anatomic defects, and the remaining 16 pregnancies were diagnosed with intra-uterine growth restriction (IUGR) as well. All CMA tests were normal, except for one pathogenic finding in the IUGR group - a 16p11.2 duplication sized 722Kb. Literature search yielded 394 titles, of which two relevant articles were found. One of these, published at 1998, yielded 31 (20.1%) abnormal karyotypes in 154 pregnancies with oligohydramnios (11 of these as an isolated findings), while another (1995) did not find any aberrations in 28 pregnancies with isolated oligohydramnios.

Discussion: Current evidence does not support invasive prenatal testing in pregnancies with isolated oligohydramnios.

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P01.57A
Early experiences with screening for aneuploidy in preimplantation genetic testing for inherited disorders

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Preimplantation genetic testing (PGT) is divided into three categories based on the indication: PGT-M (Monogenic), PGT-SR (Structural rearrangements) and PGT-A (Aneuploidy). A large study of more than 15000 trophectoderm biopsies found the lowest rate of aneuploidy to be 25% in young women increasing with maternal age to more than 90%. Thus at least 1 in 4 embryos are expected to be aneuploid, possibly leading to implantation failure, miscarriage or an abnormal fetus. Hence, selection of euploid embryos should in theory increase pregnancy rates and decrease miscarriage rates, but this is still a subject of intense international discussion. In our center we don't offer PGT-A, but as our PGT-SR setup is based on Shallow Whole Genome Sequencing, the ploidy status is revealed, and thus used for prioritizing embryos. Our PGT-M setup includes fragment analysis of short tandem repeats, and SNaPshot analysis of the specific mutation when relevant. Since PGT-M setup does not detect aneuploidy, we would expect a higher rate of implantation when performing PGT-SR. Mean maternal age is similar in the two groups (30.96 in the PGT-M and 32.56 in the PGT-SR group). We

examined the rate of positive hCG and ongoing pregnancies (OPR) per transfer (SET) on embryos (159 PGT-M and 27 PGT-SR) transferred between 01-01-2017 and 01-12-2018. Surprisingly, we found no significant difference in outcome in the two groups in terms of positive hCG (49.7 vs. 48.1, P = 0.881) or OPR (34.6 vs. 37.0, P = 0.805), but the number of cases is still rather low.

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P01.58B
Counseling conundrum: sex discordance identification following preimplantation genetic testing or noninvasive prenatal testing using SNP-based methodologies

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Introduction: Preimplantation genetic testing (PGT) and noninvasive prenatal testing (NIPT) can determine fetal sex with high accuracy prior to/during pregnancy. However, additional prenatal screening or clinical presentation may indicate sex discrepancy requiring medical follow-up for an infant's health.

Methods: Cases of possible sex discrepancy after PGT or NIPT at a single lab were retrospectively reviewed. For PGT, genotyping was performed using Illumina Cyto12 SNP-based microarray with informatics. For NIPT, cell-free DNA was isolated and amplified by massively-multiplexed PCR targeting 13,392 SNPs covering chromosomes 13, 18, 21, X and Y. Only cases with testing to identify a cause for discrepancy were included.

Results: Four of 23,297 (0.02%) PGT and 49 of 1,081,541 (0.005%) NIPT cases had discrepant sex by prenatal screening or postnatal exam. For PGT, child concordance with parental samples and remaining embryos revealed 2 (50%) resulted from incorrect embryo transfers and 2 (50%) resulted from natural conception around the time of embryo transfer. For NIPT, phlebotomy labeling errors comprised 6 (12.2%); confined placental mosaicism, 10 (20.4%); ultrasound errors, 13 (26.6%); and disorders of sexual development (DSD), 20 (40.8%). No discrepancies were due to lab error for PGT/NIPT cases.

Conclusions: Causes of discordant sex after PGT or NIPT can include sample swap, ultrasound errors, PGT/NIPT result errors, natural conception around the time of embryo transfer, vanished twin on non-SNP-based NIPT, embryo mosaicism, confined placental mosaicism and various DSDs. A thorough investigation can provide reassurance and guide appropriate medical management and counseling about cause and recurrence risk.

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P01.59C
Preimplantation genetic testing of monogenic disease: experience in Russia

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Introduction: Preimplantation genetic testing of monogenic disease (PGT-M) is an alternative to prenatal testing for couples with high risk of having offspring with single-gene disorder. PGT-M is technically challenging, because of extremely small amount of biomaterial. We report here our experience of PGT-M in "Genetico" center in Russia.

Material and Methods: A retrospective analysis of all requests and cycles of PGT-M referred to our center was performed. Personalized PGT-M assays combine direct diagnosis of the pathogenic variants and linkage analysis of highly heterozygous STRs. PGT-A by NGS or aCGH was performed for unaffected embryos upon patient's request.

Results: Of 109 couples referred to our center for PGT-M, 92 completed preliminary test for PGT-M for 42 genetic condition: 24 autosomal-dominant, 54 autosomal-recessive, 13 X-linked requests. The most frequent indication was spinal muscular atrophy (17). We performed 85 PGT-M cycles with 413 embryos. The whole genome amplification failed in 16 cases (3,9%). Median number of markers in test-systems was 12 and for embryo analysis it was 10. These highly informative test systems contributed to low number of inconclusive results - only for 7 samples (1,8%). For 156 (58,6%) unaffected embryos PGT-A was performed and 91 (34,1%) were suitable for transfer. At the moment we have

information about 43 transfers, 19 pregnancies and 7 healthy births and no affected pregnancy or birth.

Conclusions: Highly informative test system and accurate analysis of results can lead to both - high accuracy of obtained results and decreased number of embryos, that were rejected because of inconclusive results.

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P01.60D
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. 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 (n = 40), implicated in PLINK v1.07 (Purcell et al 2007). microRNAs and their eQTLs were cross-referenced with newbors growth parameters. Additionally association testing between identified miRNA eQTLs and placental expression levels of predicted target genes were analyzed. 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). eQTL and newborn growth parameter association was validated in combined REPRO-META and Happy Pregnancy cohort (n = 2100)

Results: In total, 11 placental microRNAs were detected that were expressionally modulated by eQTLs. Four of these microRNAs and their eQTLs show assosiation with newborns growth parametes. Several novel target genes and biological pathways were identified for these microRNAs.

Conclusions: miRNA eQTLs may represent additional modulators of the placental transcriptome, placental function and pregnancy course.

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P01.62B
Whole exome-based preconception carrier test (PCT) for consanguineous couples: first results from clinical practice

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Introduction: consanguineous partners are at increased risk of both being carrier of the same autosomal recessive (AR) disorder, with 25% risk of affected offspring as a consequence. Until recently, no comprehensive preconception carrier test was available to identify the very rare disease-causing mutations these couples may carry. We developed such a test for consanguineous couples and implemented this in our clinical practice.

Materials and Methods: both individuals of a couple undergo whole exome sequencing (WES). First the couple's sequence data are merged: only variants present in the same gene in both of them and with a population frequency <5%, remain in the dataset. Subsequently, this dataset is filtered against a gene panel, consisting of >1900 genes associated with known AR diseases (OMIM-based). Only combinations of likely pathogenic or pathogenic mutations (class IV or V variants) in both partners are reported. Carriership in only one individual is not detected.

Results: thus far 22 consanguineous couples were included. For 7 (32%), 'unexpected' pathogenic variants were reported conferring risk of severely affected offspring, allowing these couples to opt for prenatal or preconception diagnostic choices. Disease examples are: sulfite oxidase deficiency, AR epidermolysis bullosa dystrophica, restrictive dermatopathy, infantile epileptic encephalopathy. None of the disease associations were linked to any known disease in the family, demonstrating the innovative value of this approach.

Conclusions: our WES-based preconception carrier test (PCT) provides a powerful diagnostic tool for identification of serious disease carrier status in consanguineous couples. Outcomes provide significant reproductive choices that no other test currently offers.

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P01.63C
CarrierTest - the expanded preconception carrier screening

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We have developed a NGS panel which screens (i) 889 mutations causing 68 severe genetic disorders that can affect progeny of healthy individuals (genetic compatibility test), (ii) mutations associated with blood hypercoagulability influencing infertility treatment and pregnancy, (iii) inherited ovarian response to gonadotrophin stimulation. The test is designated for patients undergoing an IVF program and for gamete donors.

CarrierTest is a custom NGS panel with locally assembled bioinformatic pipeline and variant database. To replace MLPA and fragmentation analysis we implemented specific design adjustments and sequencing data analysis allowing detection of large deletions of *SMN1, CFTR* genes and also microdeletions on chromosome Y (AZF region) within one laboratory test. The report contains comparative analysis of detected variants for evaluation of residual risk and preconception compatibility of couple for consideration of preimplantation (PGT-M) or prenatal diagnostics.

So far 8258 samples were analyzed. These include: 3196 couples before conception, 1388 gamete donors and 478 patients with reproduction impairment without compatibility testing. Frequent occurrence of carriers was observed in the commonly screened genes (*SMN1 2,4%, CFTR 3,7%, GJB2 6,2%*) but also in other genes previously not tested (e.g. *ABCA4* 4,2%, *DHCR7* 2,7%, *SERPINA1* 2,7%, *PAH* 2,5%, *ACADM* 1,5%, *ATP7B* 1,2%, *AR* 1,1%). We identified 79 pairs (2,4%) with a reproduction risk, which is twofold increase detection rate in comparison with only *CFTR/SMN1/GJB2* testing.

CarrierTest facilitates informed decision about reproduction. Screening of couples and gamete donors allows to elucidate higher number of carriers of severe disorders in order to offer appropriate preconception and prenatal care.

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P01.64D
The prevalence of genetic and anatomic fetal defects: data from Happy Pregnancy Study

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Introduction: The study aimed to assess the prevalence of genetic and anatomic abnormalities among the participants of the prospective Happy Pregnancy study ("Development of novel non-invasive biomarkers for fertility and healthy pregnancy").

Methods: The pregnancy course and outcome including the first trimester serum test and ultrasound scans at 11-14 and 19-21 weeks were documented in the cohort of 2320 women visiting the Women's Clinic of Tartu University Hospital, Estonia in 2013-2015. Targeted FISH, karyotyping and SNP-based array was applied in cases of abnormal findings after 12 gestational weeks.

Results: In 63 cases (2.7%), the pregnancy resulted with early (<12 weeks) /late (>12 weeks) loss (n=42 /8) or termination of pregnancy (TOP) before 22 weeks (n=13). The genetic disorders were detected in 16 cases (0.7%), the anatomic defects occurred in 53 cases (2.3%). Serious anatomic defects, resulted with TOP, fetal death or postnatal surgical intervention during the first year after birth occurred in 14 cases (0.6%); anomalies two were not antenatally diagnosed: univentricular heart and cleft lip.

Defect	Antenatal diagnosis	Diagnosis after birth
Genetic	9 (7)*	7 (1 trisomy 18)
Hand/foot	1	7
Cleft lip/palate	1 (1)*	1
Cardiac mild/severe	1/4 (1)*	7/1
Urogenital	12	5
Gastrointestinal	4 (1)*	1
Cerebral	4 (1)*	0
Minor	1	3
Total	37 (11)*	32

*termination of pregnancy

Summary: The overall prevalence of the serious genetic and/or anatomic among unselected pregnant women in Happy Pregnancy cohort was 0.97%.

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P01.65A
Selecting fetuses in ambiguous setting: Preimplantation Genetic Diagnosis (PGD) for variants of unknown significant (VUS)

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The introduction of new technologies of chromosomal microarrays (CMA) and whole exome sequencing is leading to wider awareness of people to their genetic background and influence on offspring. The decision to perform PGD to select an embryo with no inherited VUS, considering the inherent ambiguity of VUS which is not unequivocally linked to a disorder, may give false hope and lead to frustration if an affected child is born. In order to evaluate Israeli VUS carriers' intentions regarding PGD for VUS findings, we have analyzed database from a large PGD center. Twenty three couples required PGD for VUS detected, in at least one spouse, by CMA analysis. Five couples had two VUS detected. Eight couples (35%) had previous pregnancy termination (TOP) solely due to the detection of VUS in an embryo. Thirteen couples (57%) had no other indication for PGD. The interpretation of VUS in the time of counseling was 'likely-pathogenic' in 10 cases (36%), 'VUS' in 9 cases (32%) and 'likely-benign' in 9 cases (32%). PGD was performed in 14 couples (61%): 9/10 likely-pathogenic, 5/9 VUS and 1/9 likely-benign VUS. Although most couples performed PGD for a likely-pathogenic VUS, some couples regard likely-benign VUS as an indication for TOP and PGD. Future demand for such controversial PGD applications will be growing and challenge the medical milieu also considering continuous re-interpretation of VUS. Setting guidelines for VUS' interpretations, proper counselling regarding VUS' meanings and defining the justified applications of PGD uses are crucial steps in practicing PGD for VUS findings.

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P01.67C
Preimplantation genetic test in couples with history of infertility and pregnancy loss

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Introduction: Preimplantation genetic test (PGT) is a cutting-edge technology of early genetic disease detection in embryos prior to their implantation in the uterus. The purpose of our study is to show the value of PGT in couples with infertility; its ability to reduce the risk of birth of chromosomally unbalanced offspring and pregnancy loss; to increase the chance of successful pregnancy and birth of an unaffected child.

Materials and Methods: We describe 185 couples with a history of infertility and pregnancy loss, who after extensive genetic counseling opted for in vitro fertilization procedure with PGT. Trophectoderm biopsy was carried out on 497 blastocyst stage embryos originating from 231 oocyte retrieval cycles. DNA was extracted and subjected to whole genome amplification. Array-based comparative genomic hybridization with microarray platforms 24sure v3/24sure+ and next-generation sequencing with VeriSeqPGS LibraryPrep protocol were performed. Results were analyzed by BlueFuse Multi version 4.3 software.

Results: 196 embryos had a balanced profile (39.44%), 292 embryos showed an unbalanced profile (58.75%) and 9 embryos could not be interpreted (1.81%). Embryo transfer was conducted in 109 cases (58.92%) and chemical pregnancy was detected in 33.03% of the women. Pregnancy loss occurred in 3.67% of cases. Live birth rate was 29.36%.

Conclusions: PGT reduces the number of failed transfers and eliminates the trauma of terminating desired pregnancy and possible medical complications. In couples with reproductive failures, PGT can be recommended in order to considerably increase the chance of conceiving with a chromosomally balanced embryo and live birth of a healthy offspring.

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P01.68D

Rapid whole exome sequencing to identify the underlying genetic cause in fetuses with sonographic anomalies

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Rapid whole exome sequencing (rWES) in critically ill newborns with a presumed genetic disorder has shown to increase diagnostic yield earlier in life, which leads to improved patient care. Due to this impact, rWES has become a routine genetic test in this group of patients. We hypothesize that rWES could have the same effect even before birth. Therefore we studied the use of rWES in fetuses with a wide range of sonographic anomalies representing the clinical practice.

We performed a retrospective analysis of the first 54 cases referred to our laboratory for prenatal rWES following the detection of fetal sonographic anomalies. Parental and fetal DNAs were sequenced simultaneously in 53 of the cases and as singleton analysis in one case due to a deceased parent. The most common identified sonographic anomalies were (multiple) congenital anomalies, skeletal dysplasia and intracerebral structural anomalies.

We were able to identify a causative pathogenic molecular variant in 12 of the 54 cases (22%) and a likely causative pathogenic molecular variant in another five cases (9%), bringing the molecular diagnostic yield in this cohort to 31%. Pathogenic and likely pathogenic variants were detected in fetuses with skeletal dysplasias (n=11, 65%), multiple congenital anomalies (n=4, 23%) or intracerebral structural anomalies (n=2, 12%).

These results suggest that rWES is likely to improve prenatal diagnosis of fetuses with ultrasonic abnormalities.

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P01.69A

Half decade experience: karyotyping, aCGH or NIPT - changes in prenatal testing strategy

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Over 5 years the strategy of genetic counseling significantly has changed after introduction of new prenatal diagnostic methods of detection chromosomal abnormalities. In 2013 our laboratory had possibility to use cell-based FISH and karyotyping for pregnancies at risk (maternal serum screening, advanced age, ultrasound examination (US), family history, patient's anxiety). Since 2014 patients in first trimester with NT>=2,5 mm or/and US abnormalities were recommended chorionic villus sampling followed by karyotyping and DNA extraction which allowed to perform aCGH (array-based comparative genomic hybridization) in case of normal fetus karyotype. In second trimester after the amniocentesis mostly aCGH/FISH analysis were recommended due to time limit with compare to cell cultivation of amniotic fluid for standard karyotyping. Chromosome imbalances by aCGH let us reveal about 20 families where one of the parents had a balanced rearrangement invisible by standard karyotyping. For these aims subtelomeric FISH analysis have been performed. When a noninvasive prenatal testing (NIPT) appeared in 2016 the whole amount of invasive procedures decreased up to 50%. NIPT should be recommended to women without US abnormalities and family history (Table). Otherwise only invasive prenatal testing is acceptable due to limits of NIPT. Since 2013 more than 12000 prenatal analyses were done. aCGH increased the detection of pathogenic chromosomal aberrations on 12%. Prenatal counseling is the most important tool for clarifying the appropriate strategy in each case.

Table. Comparison of NIPT results.

NIPT test	Result		Confirmed	False positive	False negative	Refused invasive procedure
	Low risks	High risk				
Panorama (Natera, since 2016)	3381	191	91(61%)	58 (38%)	1 (0,6%)	41
Harmony (Ariosa Diagnostics, since 2016)	1939	79	57(80%)	14 (20%)	0	8
VERASITY (NIPD genetics, since 2017)	1583	33	31 (100%)	0	0	2
Total	**6903**	**303**	**179**	**72**	**1**	**51**

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P01.70B

Correlation between reason to apply a prenatal diagnosis for aneuploidy and the results of test - A retrospective study on 2,881 foetus investigated in last 15 years by FISH method in "Cuza Voda" Maternity, Iasi, Romania

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We analysed retrospectively 2,881 cases investigated by prenatal diagnosis, using FISH method in the last 15 years in Iasi, Romania. We used probes for chromosomes 13, 18, 21, X and Y and we found 215 foetus with aneuploidy (7.46%). In 642 cases with abnormal double test we identified 17 chromosomal anomalies [2.64%] (12 cases with trisomy 21 and 1 case with trisomy 18, trisomy 13, trisomy XXY and trisomy XYY). In 577 cases with abnormal triple test we identified 8 chromosomal anomalies [1.38%] (4 cases with trisomy 21 and 4 cases with trisomy 18). In 42 cases with abnormal double and triple test we did not identified chromosomal anomalies. In 759 cases with congenital anomalies identified by ultrasonography we found 162 chromosomal anomalies [21.34%] (78 cases with trisomy 21, 52 cases with trisomy 18, 13 cases with trisomy 13, 10 cases with monosomy X and 9 cases with triploidy). In 732 cases with advanced maternal age (> 35 years) we identified 16 chromosomal anomalies [2.18%] (14 cases with trisomy 21 and 1 case with trisomy 13, and triploidy). We applied FISH test in 11 cases with positive NIPD and we confirmed 9 cases with trisomy 21 and one case with monosomy X. In 118 cases we made prenatal diagnosis for other reason (usually for chromosomal pathology in antecedents) and we found only two cases with trisomy 21 [1.69%]. In conclusion, prenatal diagnosis of chromosomal disorders is very useful in cases with congenital anomalies and positive NIPD.

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P01.71C

Applicational of the array comparative genomic hybridization (aCGH) in the prenatal diagnostics of fetuses with increased risk of aneuploidy

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The occurrence of aneuploidy is estimated at 5 - 11 % of all fetuses. The most common are trisomies of chromosomes 13, 18, 21 and monosomy of the chromosome X. Noninvasive prenatal screening tests allow the risk calculation of the aneuploidies based on the patients age (above 35), biochemical markers (free β-hCG) and ultrasonographic markers (nuchal translucency parameter). The results suggesting a high risk of aneuploidy give strong indications for further invasive diagnostics.

The aim of this project was to evaluate the frequency of chromosome aberrations other than aneuploidies in the group of 204 patients with high risk of trisomy based on the screening tests, where the ultrasound test did not show any abnormalities. To perform the array diagnostic evaluation DNA from uncultured amniotic fluid, trophoblast or cultured amniocytes was used.

Aneuploidy was identified in 34/204 fetuses (17%). Trisomy 21 was the most frequent abnormality (66%). The trisomy 13 and 18 were detected in 3% and 11%, respectively, followed by X monosomy (11%), X mosaic monosomy (3%) and sex chromosomes disomy (11%). In 10/204 cases (5%) structural aberrations of 115 kb to 41 Mb were identified. Two of them were classified as potentially pathogenic and 8 as pathogenic, where 4 were localized in

the regions of known microdeletion and microduplication syndromes.

Therefore, we postulate that CGH is the most reliable and the fastest method for identification of genomic imbalances, even in the cohort of patients with normal results of ultrasound examination.

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P01.72D

Prenatal whole exome sequencing detects a novel Fukutin (*FKTN*) mutation in a fetus with a suspected recurrence of Dandy-Walker malformation

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Introduction: Posterior fossa malformations are among the most frequent Central Nervous System anomalies prenatally detected. We report on a 17 weeks male fetus with an ultrasonographic suspicion of Dandy-Walker Malformation (DWM) recurrence. The previous pregnancy of the couple had been interrupted at 22 gestational weeks following a diagnosis of DWM. We identified the molecular cause using Next Generation Sequencing in prenatal diagnosis.

Materials and Methods: Whole exome sequencing (WES) has been performed on fetal genomic DNA. After reads pre-processing, mapping, variant calling and annotation, a filtering strategy based on allelic frequency, recessive inheritance and phenotypic ontologies has been applied. A fetal MRI at 18 gestational weeks has been performed. In silico analysis of a potential causative variant in the Fukutin protein has been carried out through a structural modeling approach.

Results: We identified a novel homozygous missense mutation (NM_006731.2, c.898G>A, p.Gly300Arg) in Fukutin gene (*FKTN*), which is associated to Muscular Dystrophy-Dystroglycanopathies. Fetal MRI supported molecular findings. Structural modeling analyses indicated a potential pathogenetic mechanism of the variant, through a reduced activation of the sugar moieties, which in turn impairs transfer to α-dystroglycan and thus its glycosylation. These findings pointed to a redefinition of the ultrasonographic suspicion of DWM recurrence to a Muscular Dystrophy-Dystroglycanopathy type A.

Conclusions: The present case confirmed WES as a reliable tool for prenatal identification of molecular bases of Central Nervous System phenotypes. Moreover, it highlights the importance of considering Muscular Dystrophy-Dystroglycanopathies when a posterior fossa anomaly is early prenatally described.

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P01.73A

The advantage of comparative genomic hybridization (aCGH) over quantitative fluorescence PCR (QF-PCR) in genetic testing of chorions after miscarriage

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At least 60% of spontaneous abortions occur due to genetic disease (most often chromosomal aberration) in the embryo and fetus. Genetic testing of miscarriage material is very important in order to determine the etiology of miscarriage and to identify couples at risk.

In our laboratory two methods QF-PCR (for chromosomes: 13, 15, 16, 18, 21, 22, X, Y) - 827 chorions and

aCGH (with resolution 12x135k or 8x60k) - 417 chorions were used.

Chromosomal aberrations (numerical or structural) were found in 56,5% of chorions analyzed by aCGH and in 52,5% tested by QF-PCR. Among chromosome aberrations most frequent were trisomies (65,7%; most frequent trisomy 16; trisomy 14 with the same frequency as trisomy 18), 45, X (14%) and triploidy (13,1%). The higher number of miscarriages identified in an individual case, the smaller percentage of abnormal results were observed.

Structural chromosomal aberrations including CNVs were identified in 13,6% of noneuploid miscarriages examined by aCGH. Some CNVs were repeated and contained genes that could be candidate genes critical for maintaining healthy pregnancy. The most common micro-aberration (6 noneuploid chorions analyzed by aCGH) concerned the locus 3p21.31, and involved the *DAG1* gene. The *DAG1* is expressed in the placenta during pregnancy. Literature data correlate deficiency of alpha dystroglycan encoded by *DAG1* gene with embryonic lethality.

Introduction of aCGH to the diagnostics of miscarriages, enabled the dynamic development of research on genetic causes of recurrent pregnancy losses including other than chromosomal aberrations.

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P01.74B
Two years' experience with molecular diagnostics of RASopathies in prenatal cases

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Introduction: RASopathies are a group of diseases with similar symptoms caused by disruption of RAS/MAPK pathway function. The most abundant diseases are Noonan syndrome and neurofibromatosis type 1.

Materials and Methods: Resequencing of 20 selected genes was performed by massive parallel sequencing approach on samples with normal karyotype originated from amniotic fluid or chorionic villi tissue.

Results: Samples were indicated on the basis of ultrasound findings. From a total of 104 prenatal samples causative variant was found in 10 of them (detection rate 9,6 %). All those positive samples had severe ultrasound finding – increased nuchal translucency, hygroma coli, cardiac abnormalities, excess amniotic fluid, etc. Most frequently causative variants were found in *PTPN11* gene (4/10). Surprisingly, in two cases (2/10) we have detected variant in *RIT1*, one of the recently reported gene for Noonan syndrome. Especially in prenatal diagnostic variants of uncertain significance (VOUS) pose a significant problem. There is not enough information to sort them out into classificatory categories, so they complicate consecutive genetic consultation. In our group VOUS was identified in 14 samples (14/104; 13,5 %). However, segregation analysis of the variants in family members helped to clarify their significance in eight of them.

Conclusions: The aim of this study was to evaluate clinical benefits of massive parallel sequencing in prenatal diagnostics of cases suspected for RASopathy (i.e. detection rate) and compare them to the fraction of detected VOUS. In general, our results establish this approach as effective but with non-negligible number of uncertain findings.

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P01.75C
1 out of 4 healthy individuals are carriers of a severe recessive or X-linked disease after using a panel of 15 genes

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Preconceptional tests use Next Generation Sequencing (NGS) to identify carriers of recessive and X-linked diseases, with the aim of reducing the risk of offspring born with these alterations. The goal of the present study is to show the results of the implementation of a reduced carrier screening test analyzed by NGS in a population of 1097 healthy individuals who attend a reproduction center. This test includes 15 genes associated with the 16 most prevalent autosomal recessive and X-linked diseases in Caucasian population, following the current recommendations of scientific societies (high prevalence, severe, early onset and with clear genotype-phenotype relation diseases). Among the total of individuals, 809 (73.7%) are women and 288 (26.3%) are men. 25.4% are carriers of at least 1 pathogenic or probably pathogenic alteration (22.5% have a single mutation and 2.9% have two). Diseases with highest probability of being a carrier are: cystic fibrosis and

congenital bilateral aplasia of vas deferens (1/13), GJB2-related DFNB 1 non-syndromic hearing loss and deafness (1/16), familial Mediterranean fever (1/42), spinal muscular atrophy (1/50) and phenylketonuria (1/52). Out of the 809 women, 2.1% are carriers of a premutation (45-200 CGG repeats) in the *FMR1* gene, causative of X-fragile syndrome. In conclusion, 1 out 4 patients without other diseases who attend a reproduction center is a carrier of a pathogenic alteration in one of the screened genes. This test identifies the most frequent alterations that are at risk of being transmitted to the offspring to promote the autonomy of patients to make reproductive decisions.

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P01.77A

Genomic analyses in cases with reduced fertility and recurrent fetal anomalies

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Objective: Genomic aberrations are usually associated with reduced fertility, recurrent abortions or fetal anomalies. Establishment of the exact genetic diagnosis in such cases has a great impact on the determination of the reproductive risk and making a decision for reproductive options.

Design: In our study we have collected patient samples in the following clinical cases of disturbed reproduction: infertility or repeated abortions, structural fetal anomalies and intrauterine death.

Materials and Methods: We performed cytogenetic analysis on lymphocytes from couples in all the listed above cases (1425 samples) and array CGH analysis of abortion samples in cases of disturbed fetal development (40 samples).

Results: Chromosomal alterations were established by cytogenetic analysis in 93 patients (6.5%), distributed as follows - 19 chromosomal translocations (1.3%), 7 numerical X/Y aberrations (0.5%), 10 cases of X/Y mosaicism (0.7%), 2 cases of mosaic marker (0.1%), 25 cases of chr9 inversion (1.75%), 20 cases of acrocentric satellite polymorphisms (1.4%), 5 cases of pericentric heteromorphism 1qh+ and 9qh+ (0.4%), 5 structural Y-chromosome aberrations (0.4%), 2 deletions of Xqter and 1 ring21 chromosome. In cases of fetal anomalies array CGH revealed 17p13.3 microdeletion (Miller-Dieker syndrome), 1p36 microdeletion, 22q11.21 microdeletion (Di George syndrome), 22q11.1 microduplication (Cat-eye syndrome), 10q26.3 microdeletion (2 cases), 22q11.21 microduplication (2 cases), 15q11.2 microdeletion (3 cases), 17p11.2 microdeletion (Smith-Magenis syndrome).

Conclusions: In 6.5% of couples with reproductive failure chromosomal alterations were detected. There is a high probability (about 30%) to reveal microstructural genomic aberrations by array CGH in cases of recurrent fetal anomalies.

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P01.79C

Using Preimplantation Genetic Diagnosis (PGD) for retinoblastoma survivors: cost-effectiveness and quality of life improvements

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Background: Retinoblastoma (Rb) is a paediatric cancer, leading to loss of vision, eye(s) or life. Approximately 40% of patients have a heritable form of the disease, caused by a mutation in *RB1*. Offspring of these individuals are at a 50% risk of inheriting the disease. Aggressive and invasive treatments in the first five years of life under general anaesthetic, followed by regular monitoring until age 18, impact the patient, family, hospital resources. Preimplantation genetic diagnosis (PGD) offers alternative reproductive choices for individuals with a heritable mutation. Costs of genomic sequencing are rapidly declining, with increasing availability of reproductive technologies.

Methods and Materials: We undertook a cost-effectiveness study of access to PGD for survivors with heritable retinoblastoma. We modelled the cost of reproductive technology, number of affected/unaffected births,

and quality of life gains, for parental uptake rates of PGD from 0-100%. We included the costs of hospital visits from 0-18 years, and costs of IVF and PGD using three cycles (1 fresh, 2 frozen).

Results: In a cohort of retinoblastoma survivors, using in-vitro fertilization (IVF) and PGD always led to cost-savings, and quality of life improvements, even at low uptake rates. Cost-savings were $156,538 and 20.58 Quality Adjusted Life years were gained for ten couples with 50% using PGD compared to the natural pregnancy pathway.

Conclusions: IVF and PGD were always less expensive, with higher quality-of-life compared with taking the natural pregnancy approach. Affordable and accessible PGD will lead to savings for families and health systems for families with heritable retinoblastoma.

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P01.80D
Celiac disease predisposition and Recurrent pregnancy loss: HLA- genotyping

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In recent years, has been discussed a relationship between RPL and celiac disease (CD), one of the most common intestinal disorders in the small intestine affecting up to 1% of individuals in Western populations. It is known that susceptibility to CD is linked to certain human leukocyte antigen (HLA) class II alleles, especially in the HLA-DQ region. In this context, the aim of this study was to investigate the prevalence of the CD-predisposing alleles in women with RPL.

Results: The presence of appropriate HLA alleles in 32 women with RPL and 33 women of control group was determined. High CD predisposition was considered in individuals with HLA alleles DQA1*0501, DQB1*0201-0203 (encoding HLA-DQ2) and DQB1*0302 (encoding HLA-DQ8), the presence of only the alleles DQA1*0501, DQB1*0201-0203 was considered as medium-high predisposition CD and a low risk CD are related allele DQB1*0302. A significantly higher frequency HLA-DQ2/DQ8 haplotype in women with RPL compared to control was established (21.88% vs. 3.03%, $p<0.025$). The genotype HLA-DQ2 is established with a significantly higher frequency in women with RPL compared to control (34.8% vs. 6.06%, $p <0.005$). The frequency of the HLA-DQ8 genotype was almost the same in the study groups (18.75% and 12.12%, $p>0.05$), and the absence of such CD-predisposing alleles was significantly lower in women with RPL compared to control (25% and 78.79%, $p <0.0001$).

Conclusions: The study of a possible correlation between HLA-DQ2/DQ8 haplotype and RPL might suggest new diagnostic and therapeutical approaches for RPL women.

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P01.81A
Expanding our non-invasive prenatal diagnosis (NIPD) service using droplet digital PCR

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Introduction: NIPD where the mother carries a mutation is hindered by the maternal mutant background in cell-free DNA (cfDNA). Relative haplotype dosage analysis is used in clinical laboratories for NIPD of recessive and X-linked disorders, however this technique requires DNA from both parents and a proband. Droplet digital PCR (ddPCR) offers high sensitivity for quantification of cfDNA and has potential for use in carrier mothers via relative mutation dosage using only a maternal sample. We have applied this technology to sickle cell disease and 8 X-linked disorders to determine clinical utility.

Methods: ddPCR assays were designed and validated using gDNA for the pathogenic variant. Testing was performed on cfDNA from maternal plasma samples for 22 sickle cell risk pregnancies and 10 X-linked mutations. Fetal fraction was determined using three different methods. Fetal genotype predictions were generated using sequential probability ratio test, with results confirmed by invasive sampling.

Results: For sickle cell disease, correct predictions of fetal HBB genotype were generated for 11 samples, with 2 incorrect predictions and 9 inconclusive results. Incorrect and inconclusive predictions resulted from low fetal fraction and threshold positioning during analysis. For X-linked conditions, assay design has been successful and development of the analysis pipeline initiated.

Conclusions: ddPCR has the potential to be used for NIPD for maternal mutation carriers using only a maternal sample. We will report testing of over 50 additional ongoing cases to refine and validate the analysis pipeline to further extend the scope of our NIPD clinical service.

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P01.82B

Comprehensive chromosome screening of human first polar bodies and oocytes using four different whole genome amplification methods and single-cell next-generation sequencing

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Background: Aneuploidy is the most frequent type of chromosome aberration and the most significant clinically. It occurs as a result of meiotic chromosomal malsegregation during formation of a haploid oocyte by subsequent extrusion of two polar bodies: polar body first (PB1) and second (PB2). Based on meiosis mechanism, genome of a single oocyte can be deduced by analyzing its sibling polar bodies.

Material and Methods: Next-generation sequencing technology for comprehensive chromosome screening was used to assess ploidy status of the oocytes and their sibling PB1. A total of 120 single cells (60 PB1 and 60 oocytes) were subjected for whole genome amplification (WGA) using four different commercially available WGA kits and analyzed using VeriSeq PGS kit on the MiSeq sequencer (Illumina). Random samples were validated using genomic arrays (aCGH). Aneuploidy detection with the tested kits was evaluated.

Results: Aneuploidy status was determined for 112 of 120 (93,3%) samples. In the group of well-amplified PB1-oocyte pairs (33/60) a 97% concordance between the chromosomal status of PB1 and the corresponding oocyte was observed. Differences in WGA kits performance including amplification uniformity and aneuploidy calling potential were evaluated and the most optimal kit for PB1 genome WGA was selected. Results from aCGH validation of the randomly selected samples showed full consistency with the NGS results.

Conclusions: NGS-based method used for PB1 genome analysis showed a high predictive potential of PB1 in deducing ploidy status of the corresponding oocyte and is a promising method for genetic preconception testing of oocytes.

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P01.83C

What is the actual risk of couples carriers for the mutation *DHCR7*:c.964-1G>C?

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Introduction: Smith-Lemli-Opitz is an autosomal recessive disorder characterized by variable expression including multiple congenital malformations, dysmorphic features, metabolic derangement and intellectual disability. The founder mutation *DHCR7*:c.964-1G>C was introduced into the Israeli preconception screening tests for Ashkenazi Jews in 2017 since the carrier frequency of the mutation is high in this population (1.2%). Despite the high carrier rate, the disease itself is not known to be frequent in this population. Discrepancy between the carrier and disease frequency raises the question of the actual risks for affected offspring for couples detected by the screening program.

Methods: We performed a literature review of all publications available with detailed information regarding homozygous *DHCR7*:c.964-1G>C fetuses/patients were included. We also collected clinical data about the couples identified in the national screening program, such as reproductive history.

Results: Out of 31 homozygous fetuses, six died in-utero (IUFD), ten pregnancies were terminated during the second trimester and 15 children were born. All 15 died between day one till three months of age. Reproductive history of Smith-Lemli-Opitz at-risk couples showed that out of 61 pregnancies, 32 spontaneous miscarriages were reported (52%).

Discussion: Our observations support the previous knowledge that homozygosity for c.964-1G>C in *DHCR7* leads to a severe phenotype or early spontaneous abortion.

An unexpected observation was the excess of early spontaneous abortions. The reason for this is not clear and awaits further studies.

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P01.85A
Developing quantitative real-time PCR for preconception spinal muscular atrophy carrier screening in Thai population

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Introduction: Spinal muscular atrophy (SMA) is a severe neuromuscular disease and one of the most common autosomal recessive diseases in children, caused by homozygous absence of the survival motor neuron gene (*SMN1*). Heterozygous exon 7 deletion of SMN1 is defined as a carrier with the reported frequency of 1/40 to 1/50 worldwide. Clinical practice in many countries recommends SMA carrier screening for most couples at the preconception stage.

Materials and Methods: We developed a quantitative real-time PCR protocol to detect *SMN1* exon 7 deletion (SMADX7™) as a cost-effective method, aiming to perform carrier screening in 430 Thai individuals visiting preconception clinic. Validation of the method was done in 100 positive and negative controls compared to standard DHPLC method.

Results: The result of control testing was 100% consistent between real-time PCR and DHPLC. Further population screening revealed the SMA carrier frequency of 1/72.

Conclusion: Developing of SMADX7™ is an outstanding strategy for SMA carrier screening in Thai population due to good cost-benefit and rapid turnaround time within 24 hours. Carrier frequency in our cohort seemed to be lower than global frequency; however, additional subjects are required to be enrolled, aiming to obtain the national data that can represent overall carrier frequency.

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P01.86B
Detection and validation of subchromosomal aberrations detected as part of routine noninvasive prenatal testing

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Introduction: Noninvasive prenatal testing is based on analysis of circulating DNA from blood of pregnant women. Low coverage whole genome sequencing is able to detect not only most commonly screened chromosomal aneuploidies but also different subchromosomal aberrations.

Aim: Aim of the work was prospective study of detection and validation of subchromosomal aberrations of fetal origin identified as part of routine noninvasive prenatal testing.

Materials and Methods: From April 2016 till December 2018, more than 8000 samples of pregnant women using Illumina NextSeq 500 platform were analysed. Low coverage whole genome sequencing was used in combination with optimized CNV detection algorithm.

Results: In tested cohort of patients different suchromosomal aberrations were detected in 32 samples. Half of patients (16) refused confirmatory testing or we were not able to get sample or feedback from the further management of the patient. In second half of samples (16) DNA from amniocenteses were available for confirmatory testing. Of these cases 5 findings were confirmed and 11 were negative after verification analyses. In samples with detected aberrations fetal fraction varied between 10,3 and 18,26 %. The smallest reported aberration was 1 Mb and the largest one approx. 80 Mb long.

Conclusions: In this prospective study the possibility of utilization of low coverage genomic sequencing for detection of subchromosomal aberrations over the whole genome was confirmed. For proper estimations of sensitivity, specificity, NPV and PPV larger studies are necessary as

samples significantly differ in crucial factors that are fetal fraction, position and size of detected aberration.

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P01.87C
The risk of spontaneous abortion in subfertile couples

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Our research has gone from the observation that women with a history of subfertility have been shown to have increased rates of subclinical early pregnancy loss, as detected via human chorionic gonadotropin, relative to women without a history of impaired fertility.

Objectives: The purpose of this study was to assess the association between subfertility and spontaneous abortion.

Methods: A total of 122 women in Constanta reported 384 pregnancies between 2014 and 2018 and were able to provide an estimate of the waiting time to conception for 276 (71.87% of the 384 eligible pregnancies). We noted retrospective data, including sociodemographic characteristics, obstetric and medical history, genetic risk factors, cigarette smoking, caffeine and alcohol consumption, and occupational information. Rates of spontaneous abortion were determined among women with and without subfertility, and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated via multiple logistic regression.

Results: Multivariate logistic regression analyses with generalized estimating equations indicated that spontaneous abortion rates were 28.0% in pregnancies preceded by subfertility and 17.0% in pregnancies without impaired fertility (adjusted OR=1.68, 95% CI=1.36, 2.84).

The attributable risk of spontaneous abortion associated with subfertility was 6.9%.

Conclusions: Subfertile women evidence an increased number of spontaneous abortions.

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P01.88D
A molecular strategy increases the PGD accuracy for Taiwan b-thalassemia population

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β-thalassemia carries over 1.1% prevalent rate in Taiwan and has polymorphism variants coordinated with geography. Both β-thalassemia carrier couple would transmit 1/4 risk of thalassemia major to their offspring in every pregnancy. Thalassemia major patients require lifelong transfusions, iron-chelation therapy and, an expensive support system. These bring a great socioeconomic burden to the suffered family. PGD (preimplantation genetic diagnosis) for β-thalassemia is conducted in IVF (in vitro fertilization) to transfer an embryo without thalassemia major to the uterus. The problem of allele dropout (ADO) and preferential amplification highly exist in PGD involving WGA (whole genome amplification) of blastocyst biopsies. It had been reported that a combination of Sanger sequencing and QF-PCR of STR markers increased the detection rate of the mutated β-globin gene in PGD. However, the uninformative STRs and ADO of the mutated gene usually limited the validation of PGD. Moreover, the heterozygosity rate of STRs is highly population different. In this study, we verified several STRs for providing enough informative STRs for Taiwan β-thalassemia population. Our results showed that six linked STRs (D11S4146, D11S1760, D11S1243, D11S4891, D11S2352, and D11S1871) with highly heterozygous polymorphism and low recombination rate in Taiwan population and would achieve up to 99% diagnostic validation in PGD for β-thalassemia. Furthermore, the multifluorescent PCR of STRs conducted in a single tube for the linkage analysis of the mutated β-globin gene provided a simple method for performing the PGD of β-thalassemia major. This study was supported from MOST of Taiwan (107-2314-B-040-019), CSMU (104-OM-A-102), and CSMU-CCH (CSMUCCH-102-06).

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P01.89A
Maternal and paternal genotypes analysis of thrombophilic gene variants in the risk of reproductive disorders in married couples

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Introduction: Inherited thrombophilia is well defined. Factor V Leiden mutation and prothrombin G20210A genetic variants are classic thrombophilic determinants for clinical manifestation of reproductive disorders. But new genetic variants are discussed now. The aim of the study to evaluate the influence of classic and new genetic variants on reproductive disorders in married couples.

Materials and Methods: The genetic variants of *Factor V* (G1691A, rs 6025), *prothrombin* (G20210A, rs 1799963), *PAI*-I (675 5G/4G, rs 1799889), *FGB* (C148T, G-455A, rs 1800787 and rs 1800790) were analyzed in 206 couples with reproductive disorders (69 with unspecified infertility/ 137 with recurrent pregnancy loss) and 35 couples of comparison group (who gave birth to one or two healthy children). Frequency genotypes analysis was carried out without taking into account the origin (maternal or paternal) of genotypes. SNP Stats program were used for statistical analysis.

Results: There were found no significant differences for prothrombin, Factor V and FGB genes variants among investigated groups. 5G/5G genotypes were significantly decreased among couples with reproductive disorders (Table). We identified significant dominant inheritance models for *PAI-I* (5G/4G) gene (χ^2=5,16; p=0,023; OR=0,51, 95%CI: 0,29-0,88) for reproductive failure in couples.

Conclusions: We suppose that PAI gene has a beneficial effect on reproductive function due to improved anticoagulant properties of blood when genotype 5G/5G in married couples present or for embryos this genotype are predicted.

Table. Distribution of genes variants

Gene (polymorphic variants)	Genotypes	Basic group, n/% (n=412)	Comparison group, n/% (n=70)
Protrombin (G20210A)	GG	393/95,39	69/98,57
	GA	19/4,61	1/1,43
	AA	0/0,00	0/0,00
Factor V (G1691A)	GG	394/95,63	69/98,57
	GA	17/4,13	1/1,43
	AA	1/0,24	0/0,00
FGB (C148T)	CC	209/50,73	39/55,71
	CT	180/43,69	28/40,00
	TT	23/5,58	3/4,29
FGB (-455 G/A)	GG	226/54,85	40/57,14
	GA	165/40,05	28/40,00
	AA	21/5,10	2/2,86
PAI-I (-675 5G/4G)	5G/5G	82/19,90	23/32,86
	5G/4G	203/49,27	29/41,43
	4G /4G	127/30,83	18/25,71

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P01.90B
Droplet digital PCR multiplexing for fetal aneuploidy detection

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Background: Nowadays, cell-free fetal DNA (cffDNA) in maternal plasma is routinely applied for non-invasive prenatal testing (NIPT). Detection of the most common fetal aneuploidies is ordinarily performed using NGS technology. Low concentration of cffDNA in maternal circulation prevents the utilization of cheaper and simpler technology - droplet digital PCR (ddPCR). The aim of our study was to overcome this problem by target multiplexing.

Methods: Three genes on chromosome 21 and chromosome 18 were absolutely quantified in one ddPCR reaction (hexaplex). Targets on different chromosomes were distinguished using different labelling (FAM/HEX). Expected

FAM/HEX ratio for DNA with normal karyotype is 1. In case of fetal trisomy of 21 or 18, the ratio will be deflected accordingly. Artificial mixtures of euploid and trisomic DNA were used for pilot study.

Results: DNA with normal karyotype was systematically mixed with DNA with trisomy of 21 (0%; 5%; 10%; 20%; 40%); chromosome 18 was used as a reference chromosome in this case. Eight replicates for each mixture were performed. Detected FAM/HEX ratios always corresponded to the expected ratios for respective mixtures. Even the lowest representation of aneuploid DNA (5%) was correctly detected.

Conclusion: After promising results of the initial phase with the artificial mixtures, the optimized workflow will now be applied for the analysis of plasma of pregnant women. This method could be applicable for detection of trisomy 21 or 18 in the same reaction, as both trisomies together have never been observed. Supported by the Ministry of Health of the Czech Republic RVO VFN64165

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P01.91C
Clinical implementation of diagnostic whole exome sequencing for fetal multiple congenital anomalies on ultrasound

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Introduction: Identifying the cause of fetal anomalies seen on ultrasound provides important information for perinatal management, but we can diagnose only ~40% of fetuses using conventional genetic tests (QF-PCR and microarray). Whole Exome Sequencing (WES) may improve diagnostic yield, but is challenging due to uncertainties around fetal phenotyping, variant interpretation, ethical/counseling issues of incidental findings and variants of unknown clinical significance, and the requirement of short turnaround times. In this study, we implemented WES in prenatal care to increase our diagnostic yield.

Materials and Methods: We conducted a prospective study of rapid trio WES analysis next to conventional genetic tests for twenty-five fetuses with ultrasound abnormalities. Inclusion criteria were at least two congenital malformations on ultrasound or one congenital malformation in a fetus with a sib with similar phenotype, without known genetic cause. We performed trio WES analysis using a custom virtual gene-panel (~3,800 OMIM genes, excluding late-onset disease genes).

Results: We established an additional genetic WES-based diagnosis in nine fetuses, being: syndromes of MIRAGE (*SAMD9*), Zellweger (*PEX1*), Walker Warburg (*POMGNT1*), Noonan (*PTNP11*), Kabuki *(KMT2D*), CHARGE (*CHD7*); two cases of Osteogenesis Imperfecta type 2 (*COL1A1*), and one potential diagnosis in a fetus with hydrops (*MYH7*). In six out of 25 cases the WES diagnosis aided in perinatal management. The mean turnaround time was 10 (range 6-15) working days.

Conclusion: Our prospective study shows that implementing WES as a routine test in the prenatal setting is challenging, but technically feasible and has a promising diagnostic yield and significant clinical relevance.

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P01.92D
CNVs with unknown significance on chromosome 15 in prenatal samples

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Introduction: Chromosomal microarray analysis (CMA) is increasingly used in prenatal diagnostics. It provides better resolution than conventional karyotyping, nevertheless, CNVs with variable expressivity and low penetrance require a proper interpretation attitude.

Materials and Methods: More than 2200 prenatal samples (AMC, CVS) were examined at our clinic within the last two years. In 9.8% samples an aneuploidy was detected by QFPCR. The remaining samples were analyzed by CMA as a first-tier test: samples were assessed by array CGH (or SNP-array) and analyzed with AgilentCytoGenomics software. Parental samples were analyzed using CMA to specify the origin of the identified aberrations.

Results: 72 CMA analyzed samples were considered to be pathogenic (out of them 56 below karyotype resolution) while additional 112 cases as variants of unknown significance: class 2, 3 and 4 (likely benign, uncertain clinical relevance, likely pathogenic) or without subclassification. Most frequent VOUS were identified on chromosomes 15

(19), 16 (8) and X (13). Prominent CNVs were del/dup 15q11.2 (BP1BP2) and del/dup 15q13.2q13.3 encompassing significant genes NIPA1 and CHRNA7, respectively. These CNVs are associated with neurodevelopmental disorders, autism and behavioral problems. Penetrance is incomplete and expressivity varies greatly. Databases, literature search and familial genealogy did not prove definite pathogenicity. The most of these CNVs was inherited and all traceable pregnancies with normal ultrasound continued.

Conclusion: All fetuses with VOUS on chromosome 15 except one had normal ultrasound. CNVs identified on chromosome 15 except deletion of CHRNA7, were described as likely benign with respect to prevailing parental origin and normal ultrasound.

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P01.93A

Preconception carrier screening: effect of reporting variants of unknown significance in partners of carriers with clinically significant variants

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Background: Expanded preconception carrier screening (ECS) panels can identify at-risk couples for multiple diseases. ECS reports are currently limited to pathogenic/likely-pathogenic variants (PV/LPV). Variants of unknown significance (VOUS) are not reported, even though reporting VOUS is routine in genomic and chromosomal array testing in both postnatal and prenatal settings. This policy misses pairings of PV/LPV carriers with VOUS carriers (PV/LPV*VOUS), who may be at-risk. To evaluate the importance of this omission, we examined the rates and characteristics of PV/LPV*VOUS virtual matings in an Ashkenazi-Jewish (AJ) cohort, a population with well-established preconception screening. **Methods:** We analyzed 672 AJ genomes (225,456 virtual couples) for variants in different gene-panels (exons ±10bp): AJ (55 genes), pan-ethnic (168 genes) and combined (180 genes), using an ACMG classifier.

Results: Across 180 genes, we identified 4671 variants, among them 144 (3.1%) were PV/LPV and 1963 (42%) were VOUS. The proportion of matings who were PV/LPV*PV/LPV was 2.7-3.8% (depending on the panel), similar to previous reports. The proportion of PV/LPV*VOUS matings was 17-20%. Considering only exonic, non-UTR VOUS resulted in a decreased proportion of PV/LPV*VOUS matings to ≈7%. Most (65-70%) PV/LPV*VOUS matings were at risk for severe/profoundly-severe diseases, while only 6-7% were at risk for mild diseases.

Conclusions: Non-reporting of VOUS in ECS may miss at-risk couples for severe diseases. Reporting PV/LPV*VOUS couples would triple the proportion of at-risk couples, even if considering only exonic, non-UTR VOUS. Even if only 10% of VOUS are ultimately reclassified as PV/LPV, reporting PV/LPV*VOUS would increase the yield of ECS by ≈25%.

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P01.94B

Incidence of Y chromosome microdeletion in different world populations: systematic review

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Introduction: Y chromosome microdeletions in azoospermia factor (AZF) sub-regions of its long arm are the second most frequent genetic cause of male infertility. The aim of this study was to analyze data on Y chromosome microdeletion world-wide.

Materials and Methods: A recall search yielded 924 papers, 73 papers met the inclusion criteria. Risk of bias was the size of tested population (>100 men) and its application excluded 31 studies, yielding 42 papers for final analysis.

Results: The mean Y microdeletion frequency for all included studies was 8.35% (n=32996), and 6.4% (n=31552) for the studies that passed risk of bias criteria. The mean Y microdeletion frequencies per continent (Asian, American, European and African) were 9%, 6.83%, 4.45% and 3.97%, respectively. The highest frequencies were detected in South Korean (38%) and Syrian (28%) populations. The lowest frequencies were in Danish (0.7%) and Croatian (0.9%) populations. The frequencies among AZF sub-regions showed the same pattern in almost all

The frequencies of different AZF sub-region microdeletions in different world populations

Continent	Country	AZFa	AZFb	AZFc	AZFa+b	AZFa+c	AZFb+c	AZFa+b+c	Ref.
EUROPE	Denmark	0%	1.54%	**92.30%**	0%	0%	3.08%	3.08%	Olesen *et al*. 2017
		0%	0%	**66.67%**	0%	0%	33.33%	0%	Bor *et al*. 2002
	Slovakia	25%	0%	**50%**	0%	0%	12.5%	12.5%	Behulova *et al*. 2011
	Netherlands	0%	0%	**100%**	0%	0%	0%	0%	Dohle *et al*. 2002;
		0%	0%	**100%**	0%	0%	0%	0%	Paracchini *et al*. 2000
	Slovenia	10%	0%	**50%**	0%	0%	20%	20%	Peterlin *et al*. 2002
	Italy	11.11%	8.08%	**65.66%**	0%	0%	13.13%	2.02%	Ferlin *et al*. 2007
	Germany	5.26%	10.53%	**78.95%**	0%	0%	5.26%	0	Maurer *et al*. 2001
	Croatia	**100%**	0%	0%	0%	0%	0%	0%	Medica *et al*. 2005
	Turkey	3.81%	3.81%	**49.52%**	0%	0%	26.67%	16.19%	Kumtepe *et al*. 2009
		21%	25%	**42%**	0%	4%	8%	0%	Cavkaytar *et al*. 2012
	Latvia	0%	0%	**60%**	0%	0%	0%	40%	Puzuka *et al*. 2011
	UK	0%	**38.46%**	**38.46%**	0%	0%	15.38%	7.70%	Mascarenhas *et al*. 2016
	Czech	0%	0%	**62.50%**	0%	0%	37.50%	0%	Machatkova *et al*,.2002
	Sweden	0%	0%	0%	0%	0%	**100%**	0%	Österlund *et al*. 2000
	Macedonia	0%	0%	**88.89%**	0%	0%	11.11%	0%	Plaseski *et al*. 2006
ASIA	Japan	0%	30%	**60%**	0%	0%	10%	0%	Nakashima *et al*.2002
	China	4.7%	4%	**73.30%**	0%	0%	11.30%	6.7%	Zhu *et al*. 2016
		2.22%	13%	**75%**	0%	0%	9.62%	0%	Elfateh *et al*. 2014
		2.01%	4.7%	**75.18%**	0%	1.34%	14.09%	2.68%	Zhang *et al*. 2013
	India	16.67%	**33.33%**	**33.33%**	0%	0%	16.67%	0%	Abid *et al*. 2008
		22.22%	11.11%	**33.33%**	0%	0%	**33.33%**	0%	Mitra *et el*. 2008
		0%	12.5%	**37.5%**	**37.5%**	0%	12.5%	0%	Dada *et al*. 2006
	Thailand	0%	33.33%	**50%**	0%	0%	16.67%	0%	Vutyavanich *et al*. 2007
	South Korea	0%	0%	**73.64%**	0%	0%	26.36%	0%	Choi *et al*. 2013
		4.95%	7.92%	**54.46%**	0%	0%	23.76%	8.91%	Kim *et al*. 2012
	Sri Lanka	0%	0%	**100%**	0%	0%	0%	0%	Wettasinghe *et al*. 2012
	Jordan	0%	0%	**66.7%**	0%	0%	33.3%	0%	Khabour *et al*. 2014
	Kuwait	0%	0%	28.57%	0%	0%	**71.43%**	0%	Mohammed *et al*. 2007
		11.1%	0%	**55.6%**	0%	0%	33.30%	0%	Alkhalaf & Al-Shoumer, 2010
	Iran	0%	0%	**50%**	0%	0%	**50%**	0%	Saliminejad *et al*. 2012
	Pakistan	0%	0%	**100%**	0%	0%	0%	0%	Siddiqui *et al*. 2013
	Palestine	0%	0%	**100%**	0%	0%	0%	0%	Shaqalaih *et al*. 2009
	Saudi Arabia	0%	12.5%	**75%**	0%	12.5%	0%	0%	Hellani *et al*. 2006
	Israel	0%	12.5%	**87.5%**	0%	0%	0%	0%	Kleiman *et al*. 1999
	Middle East	9.09%	**42.43%**	30.30%	0%	0%	18.18%	0%	Alhalabi *et al*. 2013
AFRICA	Tunisia	0%	10%	**30%**	0%	0%	**30%**	**30%**	Rajeb *et al*. 2008
		0%	0%	**100%**	0%	0%	0%	0%	Hammami *et al*. 2014
	Morocco	0%	0%	**50%**	0%	0%	**50%**	0%	Imken *et al*. 2007
AMERICA	Brazil	8.33%	0%	**58.33%**	0%	0%	25%	8.33%	Pina-Neto *et al*. 2006
	US	2.68%	11.41%	**52.35%**	0%	0%	21.48%	12.08%	Stahl *et al*. 2010
		5%	5%	**64%**	0%	0%	17%	9%	Hofherr *et al*. 2011

studies, with AZFc being most frequent (90.5%), followed by AZFb+c.

Conclusion: Isolated AZFc microdeletion was the most frequent, except for Croatian, Swedish, Kuwaiti and Middle East populations. These results can serve as a basis for creating future guidelines for genetic diagnostics of male infertility.

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P02
Sensory disorders (eye, ear, pain)

P02.01C
PAX6 gene analysis for aniridia patients: single Lithuanian centre experience in 2017-2018 years period

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Introduction: Aniridia is a very rare congenital panocular disorder characterized by complete or partial iris hypoplasia. *PAX6* gene mutations occur in around 90% of aniridia cases. Two-thirds of aniridia cases are inherited in an autosomal dominant pattern and 1/3 are sporadic cases.

Materials and Methods: *PAX6* gene analysis was performed for patients who were diagnosed with isolated and syndromic aniridia. Their first line and/or affected family members also was analysed. The *PAX6* gene was analysed by Sanger sequencing (2-13 exones). If no point mutations were found, analysis was extended to deletion/ duplication search with multiplex ligation-dependent probe amplification (MLPA) kit (*MRC-Holland SALSA MLPA P219 PAX6 kit*).

Results: 5 families and 3 single cases were tested for *PAX6* gene mutations in 2 years period (2017 - 2018) (20 analyses altogether). Six different mutations were found in 15 (75%) cases: 1 missense mutation, 1 intragenic deletion, 4 nonsense mutations. The 2 truncating mutations (c.991C>T (p.Arg331Ter) and c.392_393delTA (p. Ile131Thrfs*15)) were newly described. *De novo PAX6* gene variant c.991C>T (p.Arg331Ter) was found for patient, who diagnosed with aniridia and doubled kidney. *PAX6* gene variant c.392_393delTA (p.Ile131Thrfs*15) was found for family members with isolated aniridia. Members of one family with various clinical presentation (unilateral aniridia and Peters anomaly, cataracts, optic nerve hypoplasia) shared missense variant of unknown significance c.422T>C (p.Leu141Pro).

Conclusions: We would like to suggest that *PAX6* gene variants c.991C>T (p.Arg331Ter) and c.392_393delTA (p. Ile131Thrfs*15) are cause of aniridia for our patients.

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P02.02D
The role of next-generation sequencing in the differential diagnosis of congenital iris anomalies

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Abnormal development of the iris is a feature of several congenital ocular malformations. Classical aniridia, the most common form, is a panocular disease characterized by iris and foveal hypoplasia, keratopathy, cataracts and glaucoma, caused by *PAX6* haploinsufficiency. The presence of variable expressivity and phenotypic overlapping with other types of anterior segment dysgenesis (ASD) hinders the genetic characterization of atypical presentations of aniridia. Here, we evaluate the usefulness of NGS to improve the clinical diagnosis of these pathologies. A cohort of 106 families was studied, including cases with classical aniridia or atypical forms (iris hypoplasia, atrophy or coloboma). The entire *PAX6* gene and other 260 genes associated with ocular malformations were analyzed using custom targeted NGS and aCGH approaches, as well as clinical or whole exome and/or Sanger sequencing. About 82% of the cases presented likely pathogenic variants or CNVs in 5 different genes. Defects in *PAX6* explain 75% of cases, including not only all characterized cases with classical aniridia, but also 30% of patients with more atypical presentations, in whom LOF or atypical missense and non-coding variants were identified, respectively. Other aniridia-like patients presented pathogenic variants in ASD-related genes or variants of uncertain significance in candidate genes. Molecular analysis of iris malformations presents high diagnostic yield not only in cases with classical aniridia (95%) but also in patients with aniridia-like phenotypes (>50%). Our work evidences the clinical utility of differential genetic studies based on NGS in patients with congenital iris anomalies.

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P02.03A

Genotype-phenotype correlations in congenital PAX6-associated aniridia

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Objectives: Aniridia is an autosomal dominant severe panocular disorder caused by pathogenic variants in the *PAX6* gene or large chromosomal aberrations affecting 11p13 region. Aniridia phenotype varies greatly. Based on the occurrence of several aniridia characteristics in groups of patients with the same type of *PAX6* mutation, genotype—phenotype correlations were analyzed.

Methods: 155 patients from 129 unrelated families were examined in the course of the earlier study, 118 patients were found to have intragenic pathogenic variants while 37 others have large chromosomal deletions. Patients were allocated into 6 groups according to the type of *PAX6* mutation: missense, nonsense, frame shifting, splicing, 3′-*cis*-regulatory region deletions, and all other 11p13 chromosome deletions. Data on six frequently observed aniridic eye anomalies were collected: complete or partial aniridia, cataract, glaucoma, kerathopathy, fovea hypoplasia, and nystagmus. Fisher's exact test with Benjamini—Hochberg correction for multiple testing was applied for 2×2 contingency tables created to compare occurrence of each ocular sign in the group of patients with one type of mutations versus the corresponding occurrence in the group with all other mutations.

Results: Only patients with 3′-*cis*-regulatory region deletions significantly more often develop a distinct and milder aniridia phenotype without kerathopathy, fovea hypoplasia and nystagmus. Identified missense and splicing mutations were associated with severe aniridia phenotype and in their consequences could not be distinguished from the loss-of-function mutations.

Conclusions: Revealed genotype-phenotype correlations in *PAX6*-associated aniridia suggests common disease mechanism for all mutations except for 3′-*cis*-regulatory region deletions.

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P02.04B

Congenital aniridia caused by new PAX6 variants involving exonic cryptic-splicing sites

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Congenital aniridia is a panocular disease, with a worldwide incidence of 1:50.000-100.000 births, characterized by iris and foveal hypoplasia and additional anomalies in the cornea, anterior segment and lens, leading to low visual acuity. Up to 90% of the aniridia cases carried monoallelic loss-of-function variants causing haploinsufficiency *of PAX6*, a highly-conserved transcriptional regulator that is critical for normal ocular development. Here, we aimed to assess functionally the implication on splicing of different exonic and intronic variants of uncertain significance (VUS), mainly located in the *PAX6* homeodomain. First, novel VUS including synonymous, missense and non-canonical splicing variants were identified in our cohort of 87 Spanish families with aniridia after classical genotyping and/or targeted next generation sequencing (NGS) of the entire *PAX6* locus. No additional pathogenic variants were found in other 250 eye developmental genes. Functional splicing assays were performed using *in silico* predictors, *in vitro* minigenes and, when available, patients-derived lymphocytes cell lines. We described new spliceogenic mechanisms for *PAX6* variants through activating different exonic cryptic donors as the most plausible cause for congenital aniridia in several families. These included an additional case of parental *PAX6* mosaicism, as confirmed by digital-droplet PCR, for a synonymous variant in an asymptomatic mother with two affected siblings. In conclusion, clinical interpretation of silent and non-canonical splicing variants represents a real challenge for the genetic counseling. Our study revealed the importance of functional studies to elucidate the role of non-coding PAX6 variants underlying ocular development.

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P02.05C

Preliminary results from a new GWAS meta-analysis point at new loci for age-related hearing loss (ARHL)

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Introduction: With the progressive ageing of world population, there is a need to further investigate the genetic base of late-onset disorders such as ARHL. With this study, we aim to increase our knowledge of this complex disease.

Materials and Methods: We performed a case-control GWAS analysis on 2663 subjects (aged >=50) coming from Italy, Northern Europe and Caucasus clinically characterized including the audiometric phenotype. Cases and controls were classified as previously described (DOI:10.3109/21695717.2014.911472). Genotype data were imputed using 1000Gph3 reference panel. Results were pooled together with METAL. Replication of results was performed with UK-Biobank data (ie. speech-in-noise phenotype). Analyses on natural selection in European populations were performed and integrated haplotype scores (iHS) on significant GWAS results were collected.

Results: Meta-analysis identified some strongly significant loci. To prioritize these genes, signals of selection were checked to evaluate the presence of genetic variants that could have some evolutionary role and a stronger effect. A significant hit on chr5 was detected in a gene/protein (|iHS|score>2) catalysing the conversion of homocysteine to other amino-acids. Signal of selection was present in North European populations, but absent in South European ones. Interestingly, literature showed a relationship between hyperhomocysteinemia induced by folate deficiency and premature hearing loss, prompting further investigation and discussion on these results.

Conclusions: As the association signal is mainly driven by Northern Italian and European populations, a population-specific haplotype involved in folate metabolism should also implicated directly or indirectly in ARHL.

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P02.06D

Multi-level evidence of an allelic hierarchy of *USH2A* variants; hearing loss, auditory processing and speech/language outcomes

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Introduction: Hearing and auditory processing are fundamental to language development. Genetic mechanisms that alter these processes may therefore have secondary effects on emergent language. In this work, we consider variations across the *USH2A* gene and characterise an allelic hierarchy associated with alternate and distinct clinical manifestations including Auditory Processing Disorder (APD), Developmental Language Disorder (DLD) and Usher syndrome.

Materials and Methods: This investigation combines whole genome sequencing data from a family affected by a severe form of APD and speech dysarthria with genetic characterisation of two large population cohorts; the Avon Longitudinal Study of Parents and Children (ALSPAC) and UK10K. To compliment human findings, a mouse model with a genetic knockout of the rodent homolog *Ush2a* was assessed for auditory processing and vocalizations.

Results: Combined human and mouse results show: (1) heterozygous *USH2A* disruptions lead to impaired low-frequency sound perception; (2) low-level hearing impairments caused by heterozygous disruption of *Ush2a* associate with persistent higher-order acoustic processing deficits and altered vocalizations in mice; and (3) risk variants of *USH2A* combined with altered low-frequency

hearing thresholds lead to significantly worse language outcomes in humans.

Conclusions: We identify a complex, interactive genetic mechanism under which variants in *USH2A* contribute to low-frequency hearing loss and alter higher-order auditory processing increasing the risk of language disorder. Since these variants are found in 0.85% of the population, we recommend comprehensive genetic screening to enable the early identification of carrier individuals alongside specific assessment of low-frequency hearing in individuals at risk.

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P02.07A
Expanding the mechanisms underlying CEP290 pleiotropy: about 4 cases

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Introduction: CEP290 is pivotal for the assembly/maintenance of primary and motile cilia in a wide range of cells. Consistently, *CEP290* mutations cause a spectrum of ciliopathies ranging from Leber congenital amaurosis (LCA10) to embryo-lethal Meckel syndrome (MKS). LCA10 manifests invariably as a congenital and dramatically severe cone-dominant disease with visual function reduced to light perception. Here, we report 4 individuals with some preserved vision despite biallelism for presumably severe *CEP290* mutations and functional analysis in fibroblasts we performed to better understand the molecular bases of CEP290 pleiotropy.

Materials and Methods: *in silico*, mRNA, protein and ciliation analyses were performed in P1 (p.Ile556Phefs*17, homozygous), P2 (p.Lys170*/p.Glu1364*), P3.1 and P3.2 (c.1824+3A>G/ p.Asn2290Lysfs*6) and controls.

Results: mRNA analysis of P1 and P2 mRNA revealed spontaneous skipping of exons encompassing the premature stop codons by mechanisms involving basal exon-skipping (BES) and nonsense-associated altered splicing (NAS) individually or in combination. Protein analysis detected small amounts of a CEP290 protein and unaltered ciliation ability compared to controls. In contrast, we detected no BES or NAS which could have enabled the production of PTC-free *CEP290* isoforms from the mutant allele carrying the c.6869dup in the fibroblasts from P3.1 and P3.2. However, we detected some correctly spliced *CEP290* mRNAs transcribed from the second allele carrying the c.1824+3A>G mutation and small amounts of the protein by Western blot analysis.

Conclusion: Here, we report that CEP290 pleiotropy involves splicing regulation through different mechanisms that can act individually or in combination and include NAS, BES and hypomorphic consensus splice site mutations.

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P02.08B
The role of HCN1 variation in circadian chronotypes

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To ascertain the genetic risk factors for morningness/eveningness phenotypes we conducted a genome-wide association analysis using 316 whole genome sequencing samples. We have directly tested the association between SNPs and MEQ (morning-evening questionnaire). We computed association test results by linear regression assuming additive allelic effects (covariates: age, gender, and the 3 PCs). We detect a large region on chromosome 5 (more than ~400 adjacent SNPs in LD, spanning ~2mb) centered within HCN1, Brain Cyclic Nucleotide-Gated Channel 1. Regional enrichment yields a p-value of < 10-99. It is highly expressed in the brain and potentially modulates excitability in the brain and responding to regulation by cyclic nucleotides, playing a critical role in shaping the autonomous activity of single neurons and the periodicity of network oscillations. It has been shown in a double-mutant mouse model that lack of HNC1 mediated feedback in rod photoreceptor cells prolongs rod responses and saturates the downstream retinal network during bright light stimulation. The risk allele is effectively correlated with lower MEQ score hence evening phenotype. The locus has been shown to be a significant (1.6e-09) eQTL for HCN1 in GTEX. HCN1 channel is responsible for the feedback on the rods regulating the dynamic range of light reactivity under dim or intermediate light conditions. We hypothesize that if this feedback is not functioning properly an individual may get saturated with even dim light resulting in misperception of the light conditions resulting

in a circadian delay. This would suggest that HCN1 variations may directly impact the ME phenotype.

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P02.09C
Clinical utility of panel-based genetic testing in infants and young children with inherited eye disease

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Introduction: Advances in ocular genetics have transformed our understanding of inherited eye diseases (IEDs) and have led to the development of powerful diagnostic tests. However, integration of these tests into routine healthcare is frequently ineffective. Providing robust evidence of benefit can accelerate implementation; for example, the adoption of a genetic test is much more likely when its clinical utility (i.e. its ability to influence management and health outcomes) has been clearly demonstrated. Here we assess the current clinical utility of genetic testing in paediatric IED.

Materials and Methods: Unrelated children (0-5 years old) with IED were retrospectively ascertained through the database of the UK North West Genomic Laboratory Hub. The cohort was consecutively collected and included individuals presenting between 09/2011 and 08/2018. All study participants were examined in tertiary paediatric ophthalmic genetic clinics and underwent panel-based genetic testing.

Results: 229 children were evaluated including 33 with albinism, 83 with bilateral paediatric cataracts, 19 with congenital glaucoma, 7 with ectopia lentis and 57 with inherited retinal disorders. The diagnostic yield was highest for albinism and ectopia lentis and lowest for congenital glaucoma. Genetic testing altered management (e.g. prevented additional investigations or led to the introduction of personalised surveillance measures or determined eligibility for treatment trials) in significant subsets of children with ectopia lentis, albinism, bilateral cataracts and inherited retinal disorders.

Conclusions: Genetic testing helped identify an aetiological diagnosis in the majority of children with IED. This prevented additional unnecessary testing and provided the opportunity for anticipatory guidance in a subset of patients that could be precisely defined.

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P02.11A
Founder haplotype bearing mutation c.1621C>T (p.Gln541*) in the *FYCO1* gene causing of autosomal recessive cataract (CTRCT18) in the Sakha Republic of Russia

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Novel nonsense mutation c.1621C>T (p.Gln541*) in the *FYCO1* gene was previously revealed by whole exome sequencing as the main genetic cause of congenital autosomal recessive cataract (CTRCT18) in patients from the Sakha Republic (Eastern Siberia). In this report we present the results of the c.1621C>T (p.Gln541*) carrier frequency analysis in 424 adult individuals without of visual impairments from 7 populations of Eastern Siberia (Russians, Yakuts, Evenks, Evens, Dolgans, Yukaghirs and Chukchi). The highest carrier frequency of this mutation was found in Yakut population (7.9%), the lowest in Evenks (1.7%) and Evens (2.0%), and c.1621C>T (p.Gln541*) was absent in Russians, Yukaghirs, Dolgans and Chukchi. Common haplotypes for c.1621C>T (p.Gln541*) were reconstructed as a result of genotyping of 6 STR markers flanking the *FYCO1* gene in 25 patients homozygous for c.1621C>T (p.Gln541*) and in 114 patients without this mutation. Common haplotypes bearing c.1621C>T (p.Gln541*) indicate the role of founder effect in the spread of this mutation in the Sakha Republic. The highest diversity of the c.1621C>T-haplotypes was revealed

in the Central Yakuts ethno-territorial group. The mutant haplotypes of the Vilyui and Northern Yakuts groups are probably derived from the c.1621C>T-haplotypes found in the Central Yakuts group. Our results suggest that mutation c.1621C>T (p.Gln541*) in the *FYCO1* gene causing CTRCT18 arose about 260±65 years ago (in the middle of the XVIII century) and spread among Yakut population as a result of founder effect. This study was supported by the Ministry of Education and Science of the Russia (#6.1766.2017) and the RFBR (18-05-600035_Arctica).

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P02.12B
Clinical Exome Sequencing reveals the genetic cause in a family with congenital cataract

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Introduction: Cataracts are the principal cause of treatable blindness worldwide. Inherited congenital cataract shows all types of inheritance patterns in a syndromic and non-syndromic form. Congenital Cataract is an opacification of the lens, usually diagnosed at birth and without proper treatment, the vision never develops normally. In this study we present a family from Central Greece with six affected members born with Congenital Cataract, five males and one female, all of which had bilateral nuclear cataracts as newborns. Despite efforts to medically improve their condition, many members had a gradual deterioration of their vision which led to blindness, before the age of thirty years old.

Materials and Methods: Clinical Exome Sequencing (CES) was performed with the Sophia Genetics Protocol on a MiSeq Illumina platform, in one of the affected probands. In order to confirm our finding and to investigate the carrier status of most family members, Sanger sequencing was performed for the region of interest.

Results: The analysis revealed a possibly pathologic mutation -c.3808C>T(p.Gln1270*) -on the *NHS* gene, which is located on the X chromosome. The mutation has not been previously recorded in any database but the *NHS* gene is known to be the cause of X-linked Congenital Cataract - Nans Hooran Syndrome.

Conclusion: CES of related subjects from an isolated family with Congenital Cataract identified a causal NHS mutation and confirmed the mode of inheritance in the family as X-linked recessive. This finding will aid dramatically the clinical management of this whole family.

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P02.13C
A heterozygous likely pathogenic variant in MAF transactivation domain as a cause of bilateral congenital cataract without intellectual disability or seizures

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Introduction: Congenital cataract is a developmental disorder of the lens resulting in lens cloudiness or opacities. The hereditary congenital cataracts are genetically heterogeneous and can be transmitted under an autosomal dominant, recessive or X-linked inheritance model. The MAF gene (OMIM*177075) pathogenic sequence variants in the c-terminal DNA binding domain have been shown to cause autosomal dominant isolated congenital cataract phenotype. On the other hand, MAF N-terminal transactivation domain genetic variants are associated with Ayme-Gripp syndrome (OMIM#601088), in which the eye anomalies are accompanied by intellectual disability, sensorineural hearing loss, seizures, distinctive facial appearance, reduced growth and skeletal anomalies.

Materials and Methods: A female patient, law graduate by profession, was referred to genetic consultation in her first pregnancy for her history of bilateral congenital cataract. Other medical history was unremarkable, except for mild delay in achieving developmental milestones and mild unilateral sensorineural hearing loss. She never needed hearing aid. General examination at 30 years showed normal height and distinctive flat facial appearance. Clinical exome sequencing was used to test for causative variants in 110 known genes associated with isolated and syndromic cataract.

Results: A heterozygous likely pathogenic de novo missense variant c.188C>G (Pro63Arg) in the N-terminal transactivation domain of the *MAF* gene was identified.

Conclusions: This is the first report of pathogenic variant in the N-terminal transactivation domain of the *MAF* gene in an individual without intellectual disability/learning difficulties, seizures or significant hearing loss.

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P02.15A

Important contribution of *STRC* copy number variations to the development of mild-to-moderate hearing loss

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Background: Copy number variations (CNVs) are common cause of genetically determined hearing loss (HL). CNVs are frequently found in the *STRC* gene which is related to autosomal recessive HL. Due to presence of *STRC* pseudogene and technological limitation of CNVs detection, large deletions and duplications encompassing *STRC* are still an underestimated contributor to HL development.

Material and Methods: A group of 50 patients with mild-to-moderate HL diagnosed before the age of 10 and with no DFNB1 pathogenic was recruited for the study. All patients were tested for CNVs in *STRC* gene using quantitative comparative fluorescent PCR (QF-PCR) with primers specific to the *STRC* gene and its pseudogene followed by multiplex-ligation probe amplification (MLPA).

Results: Genetic prescreen with QF-PCR revealed a complete deletion of the *STRC* tested regions in 24% (24/100) and partial deletion in 5% of the studied alleles (5/100). This method allowed us also to identify two complete and two partial deletion of tested regions in the *STRC* pseudogene. Complete deletions of the *STRC* gene regions were fully confirmed by MLPA. One additional *STRC* partial deletion and one duplication were also detected. Considering the genotypes, in 26% (13/50) of HL patients homozygous or compound heterozygous CNVs were detected. In 10% (5/50) of patients only simple heterozygous CNVs was found.

Conclusions: This study shows that CNVs in STRC are a frequent cause of mild-to-moderate hearing loss in Polish patients. There is a strong need to include CNVs analysis of *STRC* gene in the standard HL diagnostic workflow.

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P02.16B

Genetic analysis of 320 Iranian patients affected by deafness using Sanger sequencing and Next Generation Sequencing (NGS) methods

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Introduction: Hearing loss (HL) is one of the most common congenital impairments which occurs one in every thousand children. More than half of these cases are caused by genetic factors. At the present time, more than 150 genes have been identified to be associated with hearing loss.

Materials and Methods: In this study, for more than 600 patients affected by deafness, genetic counseling was done. 320 patients selected for genetic analysis. In first step, for all selected patients, analysis of GJB2 gene was done. Then, for patients who had normal result for GJB2 gene, hearing loss genetic panel (including 127 genes involved in deafness) was performed using NGS method. Finally, for patients for whom no pathogenic mutation was detected in their genetic panel, Whole Exome Sequencing (WES) was done by NGS method.

Results: 19 different pathogenic mutations were found GJB2 gene in 92 out of 320 patients (28.75%). Deafness genetic panel and WES were performed for 65 and 57 patients with normal GJB2 test respectively. Pathogenic and likely pathogenic mutations were detected in 75 subjects including 25 novel mutations.

Discussion: Due to the prevalence of consanguine marriages in Iran, autosomal recessive hearing loss is common in our country. Genetic analysis, especially WES can be very helpful in preventing the birth of deaf children.

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P02.17C

Gene-wise burden analysis on painful diabetic neuropathy

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Introduction: Neuropathic pain is one of the most common complication of diabetic patients. It is a complex trait influenced by multiple genes and environmental factors. A debated hypothesis is that many rare variants might contribute to the missing heritability unexplained by common variants. Since frequencies of rare variants are very low, even with high penetrance, it is challenging to detect association. Burden tests collapse the rare variants within the gene as a single genetic variable. This study proposes a gene-wise burden test to look for genes with an overrepresentation of variations in painful (PDN) versus painless (PLDN) diabetic neuropathy patients.

Material and Methods: 107 candidate genes were sequenced by single molecule Molecular Probes-Next Generation Sequencing (MIP-NGS) in 61 PDN and 125 PLDN patients. To test differences in the mutational burden between PDN and PLDN, we applied SKAT-O test as implemented in EPACTS on all non-synonymous variants with a maximum minor allele frequency of 5%.

Results: Comparing the excess of rare variants in PDN respect to PLDN, *P2RX7* and *SCN11A* showed the lowest p-values (p=0.01 and p=0.03, respectively) even if they did not reach the significant Bonferroni threshold.

Conclusions: The burden approach provided biologically plausible signals that would have remained indistinguishable from random noise with the traditional single-variant analysis, because of high allelic heterogeneity. Our results could leave the possibility that a combination of protein-altering variants in *P2RX7* and *SCN11A* contribute to the risk of PDN. The work has been supported by FP7 PROPANE (602273) and the H2020-MSCA PAIN-Net (721841) Grants.

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P02.18D
Genetic causes of non-syndromic bilateral deafness in North-Eastern Slovenia

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Introduction: Mutations in the gene encoding protein connexin 26 (GJB2) have been shown as a major contributor to prelingual, sensorineural, nonsyndromic, recessive deafness. One specific mutation, 35delG, has accounted for the majority of the mutations detected in the GJB2 gene in Caucasian populations. The purpose of our study was to evaluate the characterisation and the prevalence of all, GJB2, GJB6 and other known mutations among affected individuals from North Eastern Slovenia.

Materials and Methods: There were 33 individuals diagnosed with hereditary deafness (DF) enrolled in the study. The allele specific PCR, MLPA technique and the Sanger sequencing was used to screen the GJB2 coding region. NGS sequencing with Oto-GeneSGKit® was used in 10 probands with bilateral, pre-lingual non syndromic DF with a negative medical history related to potential causes of acquired DF.

Results: The overall diagnostic yield of the DF cohort was 8/33 (24.2%). The homozygous mutation c.35delG was identified in four out of 33 patients (12.1%). By five patients mono-allelic mutation c.35delG was detected (15.15%; 5/33). Three among them were compound heterozygotes: c.35delG variant compounded with variants: c.109G>A; WTIVS 1+1: G>A; and c.235delC. NGS detected one homozygous disease-causing variant c.2464C>T in OTOF gene in one patient (10%), which is also heterozygote for the c.35delG.

Conclusion: The relative low ratio of individuals homozygous (12.1%) and heterozygous (15.15%) for the c.35delG mutation suggest that there are other genes responsible for nonsyndromic deafness in the North-Eastern Slovenian population. In one out of 10 tested probands with NGS this was confirmed.

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P02.19A
Investigating the genetic architecture of fuchs endothelial corneal dystrophy (FECD) in a genetically refined cohort of patients

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Introduction: Fuchs endothelial corneal dystrophy (FECD) is a degenerative condition affecting up to 4.5% of the population over the age of 50 years. Symptoms consequently result in a reduction in vision. An intronic CTG triplet-repeat expansion (termed CTG18.1) situated in *TCF4* has been significantly associated with disease.

Material and methods: DNA samples were obtained from British and Czech FECD patients (n=679). Samples were genotyped for CTG18.1 using a short tandem repeat (STR) assay and triplet-primed-PCR. Individuals harbouring bi-allelic non-expanded CTG18.1 alleles (<50 repeats) were analysed by whole exome sequencing (WES) and when possible followed up for familial history and deep phenotyping. WES data was interrogated for rare deleterious variants (minor allele frequency [MAF] <0.5% and CADD>20) in genes previously associated with corneal disease and genes with a defined functional role within the cornea. Samples without an identifiable rare causal variant, are currently being analysed by a gene burden analysis using an internal WES dataset as the control group.

Results: 20% (138/679) of the FECD patients had non-expanded copies of CTG18.1. Interrogation of WES data identified several candidate disease-associated variants, including COL8A2 and ZEB1 variants in atypical early-onset cases. Results of the gene burden analysis presented a number of potentially novel genes within the refined patient cohort.

Conclusion: This study reveals the incidence of non CTG18.1-mediated FECD in our cohort and aims to provide insight into the genetic causes of disease within this genetically refined patient group. This project is funded by the National Eye Research Centre and Rosetrees Trust.

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P02.20B
Clinical manifestations and novel *SOX10* pathogenic variants in Danish probands with profound hearing impairment and malformations of the semicircular canals

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Introduction: Waardenburg- and CHARGE syndromes are associated with overlapping clinical manifestations such as hearing impairment and malformations of the semicircular canals. The syndromes are heterogeneous, both with respect to the genes involved and the clinical abnormalities found.

Materials and Methods: Eleven patients from eight families were shown to have bilateral profound hearing impairment with variable additional clinical features. Complete or partial agenesis of the semicircular canals gave suspicion of either CHARGE or Waardenburg syndrome.

Results: Genetic testing was carried out which included genes involved in these two syndromes. Here we present eight families harboring *SOX10* pathogenic variants. The patients demonstrated a spectrum of *SOX10* mutations of which six were novel, six occurred de novo and two were found to be dominantly inherited. Both familial cases presented with novel *SOX10* mutations.

Conclusions: The results presented here add six novel pathogenic variants, one missense and four frameshift variants, to the spectrum of *SOX10* mutations and associated clinical features involved in Waardenburg syndrome are described. Grant references: University of Tromsø - The Arctic University of Norway.

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P02.21C
Targeted Next-Generation Sequencing (NGS) gene panel testing in 200 Danish individuals with primary non-syndromic hearing impairment

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Introduction: Hearing impairment is the most common sensory impairment and is genetically heterogeneous. Identification of the causative variants underlying hearing impairment is challenging, since >100 different genes for

non-syndromic hearing impairment have so far been reported.

Materials and Methods: In this study, 200 index patients, mostly of Danish origin, with hearing impairment underwent targeted Next-Generation Sequencing (NGS) using hearing impairment gene panels for Illumina platforms. Causative variants were confirmed by Sanger sequencing and segregation analysis was performed when relevant samples were available. Prior to NGS sequencing, DFNB1 and SLC26A4-related hearing impairment had been excluded by Sanger sequencing in most patients.

Result: Of the 200 patients sequenced, we were able to detect causative variants in 73 cases (37%). Furthermore, in an additional 12 cases (6%) only one pathogenic variant was identified so far. The study emphasizes the genetic heterogeneity of hearing impairment since causative variants were found in 41 different genes. In total, 113 different likely pathogenic or pathogenic variants were detected. Forty-four variants (38%) were novel (absent from the literature).

Conclusions: Targeted Next-Generation Sequencing allowed us to detect causative variants in 73 of tested cases. In this cohort, the diagnostic yield was slightly higher in cases with presumed autosomal recessive hearing impairment and in patients with an early onset of hearing impairment. Copy number variation detection studies are ongoing. Regarding the non-solved cases, selected families will be analyzed by whole exome sequencing.

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P02.22D

First reported Northern European with *CABP2*-related non-syndromic hearing loss

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Introduction: Autosomal Recessive Deafness 93 is caused by homozygous mutations in the *CABP2* gene located at 11q13.2. Five families with *CABP2*-related Autosomal Recessive Deafness 93 have been reported in PubMed. Four families (three Iranian and one Pakistani) are homozygous for the NM_016366.2:c.637+1G>T mutation and one Italian family is homozygous for the c.466G>T, p.(Glu156*) mutation.

Materials and Methods: We report an eight-year-old boy of non-consanguineous Danish Caucasian parents with prelingual bilateral moderate sensorineural non-syndromic hearing loss. Both parents had adult onset non-syndromic hearing loss and several members of the mother's family also had early-onset hearing loss. Diagnostic whole-exome sequencing was performed using DNA from the index on the NextSeq 500 (Illumina Inc., San Diego, CA) using SureSelect CRE Exome Kit v2 (Agilent Technologies Inc., Santa Clara, CA). Data analysis was performed by an in-house pipeline limited to 123 known hearing loss genes using VarSeq version 2.0.2 (Golden Helix Inc., Bozeman, MT). The mutation was verified by direct sequencing.

Results: NM_016366.2:c.637+1G>T was found in a homozygous state in the index. No other causative variants were identified.

Conclusion: To our knowledge, this is the first time the NM_016366.2:c.637+1G>T mutation is identified in a patient of Caucasian descend and the first time *CABP2*-related non-syndromic hearing loss is reported in a patient of Northern European descend. Grants: None

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P02.23A

Presumed, unknown second mutation in *GJB2* monoallelic patients is not a CNV in the DFNB1 (GJB2/GJB6) region, but it must exist

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Introduction: In almost 40% of patients with autosomal recessive hearing loss, biallelic pathogenic mutations in the *GJB2* gene are detected. For 6% patients with only one pathogenic *GJB2* mutation (monoallelic) the second is unknown. Moreover, there is a significant excess of monoallelic among hearing loss population (6%) compared to the normal hearing population (3%). Therefore we assume the second causal mutation, yet unknown, in part of monallelic hearing loss patients which explain their hearing loss. Because of the character of *GJB2* mutations, we expected to find the CNV, most likely one deletion within the critical region.

Materials and Methods: The CNV analysis was performed with SureSelect custom panel covering continuous chr13:20.735.021-21.102.144. Altogether 33 monoallelic patients were examined with use of three biallelic *GJB2* patients as controls. The CNV analysis was performed with NextGene sw.

Results: The shared 1.6kb large deletion was found in four monoallelic. Nevertheless, the deletion was not possible to confirm with use of another independent method as Sanger sequencing or PCR. Even the haplotype analysis did not reveal any common haplotype in monoallelic. Moreover, the NGS of a gene panel of 71 recessive hearing loss genes did not reveal causal mutation in other gene in group of 15 monoallelic.

Conclusion: Therefore we believe the second unknown and undiscovered mutation which contribute and cause hearing loss in the substantial part of *GJB2* monoallelic patients exist, but is probably not the CNV in the critical DFNB1 region.

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P02.24B
First missense variant in N-terminal cytoplasmic region of KCNQ4: analysis of the genotype-phenotype correlation

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Introduction: Pathogenic variants in *KCNQ4* are a well-known cause of autosomal dominant hearing loss (ADHL). There is a phenotypic variability observed in patients with different type and localization of *KCNQ4* pathogenic variants. To date, only three frameshift mutations were described in the N-terminal cytoplasmic domain of KCNQ4 in ADHL families with late-onset and pure high frequency HL.

Materials and Methods: DNA was collected from a five-generation family with progressive high frequency ADHL. High throughput sequencing on proband's DNA was performed. In family members (n=15) segregation analysis of the identified variants with HL was conducted using Sanger sequencing. Deep genotype-phenotype correlation analysis was performed using cross-sectional linear regression testing of pure tone audiometry results.

Results: Genetic testing revealed a novel, probably pathogenic c.274G>A (p.Glu92Lys) variant in *KCNQ4*, which fully segregated with HL in the studied family. Detected variant has not been reported in population databases and was classified as pathogenic. The p.Glu92Lys is the first missense variant identified in the N-terminal cytoplasmic region of KCNQ4. HL observed in the analyzed family was more severe at mid frequencies as compared to the previously published families with truncating variants located in this domain.

Conclusions: Identification of *KCNQ4* p.Glu92Lys in a ADHL family confirms the association between missense *KCNQ4* pathogenic variants and a high frequency HL with similar annual progression at mid and high frequencies. The data suggest that the type of *KCNQ4* detected variants provides a better prognostic factor than their topological localization.

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P02.25C
Genetic basis of autosomal dominant hearing loss in pediatric patients

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Introduction: Autosomal Dominant Hearing Loss (ADHL) is the second most common form of inherited hearing loss with an onset after the second decade of life. Current knowledge on the genetic aspects of ADHL in Polish

patients is limited, which significantly affects the diagnosis, genetic counselling and prevents prediction of disease progression.

Materials and Methods: Thirteen families with ADHL diagnosed before 18 years of age were enrolled in the study. DNA was isolated from peripheral blood or oral cavity swab samples from probands and family members. High-throughput genetic analysis using the TruSight One panel (Illumina Inc.) and the MiSeq sequencer was carried out for the probands. To confirm the presence of identified genetic variants and their segregation with ADHL in individual families Sanger's sequencing was performed.

Results: Genetic cause of ADHL was identified in approximately 60% (8/13) of the families. The identified variants were located in *ACTG1*, *COCH*, *DIAPH1*, *EYA4*, *KCNQ4*, *PTPRQ*, *TBC1D24* and *TMC1*. Among the identified variants approximately 60% (6/8) were novel. In the remaining families the selected variants did not segregate with ADHL.

Conclusions: Our results show high genetic heterogeneity of ADHL in Polish pediatric patients. Considering frequent identification of new genetic variants, it is necessary to perform thorough clinical examination and segregation analysis of the selected variants with ADHL in the largest possible number of family members. In patients with no genetic cause identified, the study area should be extended and include all protein coding regions or whole genome.

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P02.27A

Novel variants in known genes - results of genetic testing in families with autosomal dominant hearing loss

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Introduction: Hearing loss (HL) is the most common disability of human senses and genetic factors play an important role in its development. Autosomal dominant HL (ADHL) is usually characterized by postlingual age of onset and progression. To date 63 loci with 45 different genes were causally involved in the pathogenesis of ADHL.

Materials and Methods: Multigenerational families with ADHL were recruited in the study (n=19). Genomic DNA was isolated from whole blood and buccal swabs samples. In probands' DNA samples a multigene high throughput sequencing was performed. Family segregation analysis of the identified variants was conducted using Sanger sequencing. All detected variants were analyzed in the context of population databases and literature. Pathogenicity of identified variants was predicted by different computational approaches.

Results: Genetic testing revealed probably pathogenic variants in almost 63% (12/19) of the analyzed families. The majority of identified variants were novel, previously not reported and hitherto not linked to the disease (7/12). Novel variants were missense changes and all of them (except for two genetic changes located in *MYO6*) were found in different genes.

Conclusions: Our study revealed a high involvement of novel probably pathogenic variants in the development of ADHL and confirmed a high heterogeneity of the identified genetic changes. High throughput sequencing in HL patients generates large amount of data that should be interpreted carefully and confirmed by family studies. There is also a need for functional validation of the detected novel variants.

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P02.28B

Cochlear implantation outcome in patients with DFNB1 *locus* pathogenic variants - implications for precision medicine

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Introduction: Almost 80% of children with profound prelingual hearing loss (HL) have a genetic cause of deafness; most often two *GJB2/GJB6* (DFNB1 *locus*) recessive pathogenic variants. Cochlear implantation (CI) is a treatment of choice in profound HL patients but only few studies combine the etiology of HL with CI outcome.

Materials and Methods: Patients with profound prelingual HL who received CI before the age of 2 years and had a completed DFNB1 genetic testing were enrolled in the study (n=159). LittlEARS questionnaire and relative auditory development delay (RADD) at 6th month after CI activation were used to assess auditory development.

Results: Statistically significant differences were observed in RADD between patients implanted early (before 12 months of age) vs. late (after 12 months of age) and between patients with a short (≤6 months) vs. long (≥ 6 months) hearing aids (HAs) experience. Interestingly, in the most genetically homogenous patient group with two *GJB2* c.35delG pathogenic variants there was no statistically significant difference in RADD between patients implanted early and late and between patients with a short and long HAs experience.

Conclusions: In children with homozygous *GJB2* c.35delG pathogenic variants, application of CI before 12 or 24 months of age brings similar outcome. Children with an unknown genetic cause of HL should be implanted before 12 months of age to achieve better results in auditory development. Further studies in the non-DFNB1 group are planned to determine the link between genetic background of HL and the CI outcome.

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P02.31A

Hypermethylated genes and pathways in Polish children with high myopia

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Introduction: High myopia (HM) is an eye disorder characterized by refractive error (RE) greater than -6.0 diopters (D) with both environmental and genetic factors involved. Although a number of loci, candidate genes, and sequence variants have been identified in HM, the general genetic factor explaining the causes of HM has not been specified. The aim of this study was to unravel the role of epigenetic changes in HM which could explain the relation between HM and environmental factors.

Materials and Methods: In order to verify if the dysregulated methylation of genes could contribute to the HM development, using a genome-wide DNA methylation array, we studied 18 Polish cases (aged 4-12 years, RE between -6.0 and -15.0 D) and 18 matched controls. CpGs with the highest fold change or the highest difference in the methylation level between cases and controls were analyzed. Pathway overrepresentation analyses among hypermethylated genes were performed using ConsensusPathDB.

Results: In total, 1,541 CpGs, representing 1,745 genes, were hypermethylated (≥2.0-fold change, FDR p≤0.05, ROC-AUC ≥0.75) in HM subjects compared to controls. The most hypermethylated CpGs with more than 20% difference in the methylation level between cases and controls were located in *TIMM50*, *RAB3C*, *MICAL3*, *RWDD4A*, *XRCC2*, and *FARP2*. Enrichment analysis of hypermethylated myopia-related genes set (>5 genes shared, p≤0.01) revealed overrepresentation of genes from several pathways, including signal transduction, transcription, and signaling pathways as receptor tyrosine kinases, JAK-STAT, TGF-beta, EGF-EGFR.

Conclusions: Methylation of genes and disruption of identified pathways could contribute to HM phenotype.

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P02.32B

Diagnostic yield of whole exome sequencing-based genetic testing for patients with inherited eye diseases

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Genetic testing is essential in the diagnosis and management of inherited eye diseases (IEDs). However, IED diagnostics can be compromised by poorly validated and curated tests. We aimed to develop and validate a high quality whole

exome sequencing (WES) based platform and to assess diagnostic yield in a large IED cohort. Performance of the WES assay with bespoke clinical content, was tested through next generation sequencing (NGS) of reference samples using the Illumina NovaSeq platform. The assay was designed to cover clinically-relevant intronic and difficult-to-sequence regions, and detect copy number variants (CNVs). Based on this assay, 23 ophthalmology gene panels were curated. The assay showed high average sequencing depth (183x) and coverage (99.7% regions covered >20x), and uniform coverage over difficult-to-sequence regions, including *RPGR* ORF15. Detection sensitivity was high for SNVs (0.998), INDELs (0.97), 1-exon CNVs (0.93) and 5-exon CNVs (0.99). The Usher Syndrome Panel gave the highest diagnostic yield (>80%). The most frequently ordered 266-gene Retinal Dystrophy Panel yielded a diagnosis in 58% (of 1587) cases. Diagnoses were made in a total of 111 genes, the most common genes being *ABCA4*, *USH2A, RPGR, RHO* and *PRPH2*, as well as in newly implicated IEDs genes, and in custom targeted deep intronic regions. Approximately 3% of patients had a diagnostic CNV. A WES assay with boosted clinical content provides high diagnostic yields for IED patients. The genetic variability of diagnoses in our cohort supports the use of comprehensive NGS panels in IED diagnostics, to optimize diagnostic yield and clinical care.

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P02.33C

New insights into the phenotypic spectrum of PROM1-associated retinopathy

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Purpose: Inherited retinal dystrophy (IRD) refers to a group of progressive and degenerative diseases that affect the photoreceptor cells and lead to visual impairment. One of the genes associated with IRD is *PROM1*. Disease-causing *PROM1* variants have been associated with different phenotypes. The aim was to provide a detailed analysis of the genetic and phenotypic characteristics of the *PROM1*-associated retinopathy in a large cohort of patients.

Methods: *PROM1* screening was performed using classical molecular techniques and/or targeted Next-Generation-Sequencing in a cohort of 2216 IRD families. Copy number variation analysis was carried out in unsolved monoallelic cases. Detailed ophthalmic evaluation was performed in 25 patients.

Results: Thirty-two families presented *PROM1* variants, including likely pathogenic and unknown significance variants, 10 of which are novel. Causative variants were identified in 21 families segregating in autosomal recessive (17) or dominant (4) pattern, 3 of them with founder effect. Phenotypic analysis in 25 patients carrying disease-causing *PROM1* variants revealed clinical heterogeneity regardless of genotype. Most of the patients suffered from cone-rod dystrophy and some patients presented with macular dystrophy or retinitis pigmentosa, all presenting macular damage.

Conclusions: Our study reports the largest and comprehensive description of genetic and clinical findings in *PROM1*-retinopathy. The prevalence of *PROM1* variants that seems to reliably explain the IRD phenotype in our cohort is about 1%. This study also highlights the great heterogeneity of *PROM1*-associated phenotypes.

Regardless of the initial diagnosis of primary cone or rod loss, all patients developed a common macular involvement, a characteristic phenotypic finding of *PROM1*.

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P02.35A
Contribution of *DRAM2* gene in inherited retinal dystrophies with early macular involvement

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Introduction: Macular Dystrophies (MD) are a group of Inherited Retinal Dystrophies (IRD) characterized by the degeneration of the central retina or macula, principally formed by cone photoreceptors. Although there are more than 55 MD genes, a considerable percentage of these cases remain unsolved.

Methods: Whole exome sequencing of a cohort of IRD families was performed, analyzing a panel of 300 IRD genes in order to determine the genetic pathogenic cause. For some new mutations, functional analyzes were ruled out from patients blood samples.

Results: Three new *DRAM2* homozygous mutations were identified in three independent families: one frameshift and two splicing variants. All patients shared a singular phenotype, with an early macular involvement followed by a peripheral degeneration. However, the age of onset and the evolution of the pathology were different in each case. At a molecular level, functional studies revealed alterations in gene expression differing between patients.

Conclusions: *DRAM2* was firstly associated to IRD in 2015, and only scanty cases and 10 different pathogenic mutations have been reported to date. Thus, its function in the retina and the associated phenotypes are still much unknown. In this context, the results obtained significantly contribute to the comprehension of the role of this gene in the molecular bases of IRD, increasing the number and type of reported pathogenic variants and their functional effects, and strengthening the *DRAM2* genotype-phenotype correlations in the retina.

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P02.36B
Next generation sequencing in patients with bilateral optic atrophy: Leber hereditary optic neuropathy and beyond

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Leber hereditary optic neuropathy (LHON) is caused by missense mutations in the mitochondrial genes (mtDNA) encoding complex I subunits of the respiratory chain. However, mutations affecting genes in the nuclear genome may strikingly resemble the clinical presentation of LHON. We conducted a study to define best testing strategy using next generation sequencing in bilateral optic atrophy and inconclusive family history. Here we report on 30 unrelated individuals referred to our institution for genetic testing because of the optic nerve atrophy and clinically suspected LHON. None of the patients had lesions suggestive for multiple sclerosis on brain MRI. Genetic analysis consisted of mtDNA sequencing followed by clinical exome sequencing. In two unrelated patients mtDNA sequencing identified typical LHON pathogenic variants (m.11778G>A, m.3700G>A). Exome sequencing in three unrelated patients revealed *OPA1* mutation (c.2489G>A, likely pathogenic variant), compound heterozygous mutations in *ACO2* (c.2253dupC, VUS and c.719G>C, pathogenic variant) and a de novo mutation in WFS1 (c.2480C>T, likely pathogenic variant). Upon genetically guided clinical review, the last patient was found to exhibit signs on macular OCT, typical for Wolfram syndrome. We found possible causative variants in 5 of 30 patients with bilateral optic atrophy and clinically suspected LHON. In addition to typical LHON mitochondrial variants we found causative variants associated with autosomal dominant, autosomal recessive and syndromic forms of optic neuropathy and reclassified clinical diagnoses. Our results provide evidence that a combined approach with mtDNA and clinical exome sequencing is recommended in cases of clinically suspected LHON and negative family history.

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P02.37C
Targeted next generation sequencing reveals homozygous *PDE6C* mutation in a pedigree with macular aplasia

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Introduction: Macular, or foveal aplasia refers to the lack of foveal depression with continuity of all neurosensory layers in the presumed location of the fovea. It has been reported in cases of aniridia, albinism, microphthalmia and achromatopsia. Inherited retinal dystrophies (IRD), among which macular aplasia (MA), constitute a group of diseases characterized by clinical variability and pronounced genetic heterogeneity. By using IRD-panel next-generation sequencing, we aimed to identify the disease-causing mutation in a large Bulgarian pedigree with MA inherited in autosomal-recessive pattern.

Materials and Methods: A large pedigree with MA, most likely achromatopsia, accompanied by nystagmus and decreased visual acuity presented from birth was recruited and DNA from the proband was sequenced using the Illumina® platform and TruSight One sequencing panel. Protein coding and splice-site variants were filtered in a panel with 341 genes related to retinal dystrophies.

Results: Genetic analysis identified a homozygous missense variant c.2141T>A (p.Ile714Asn) in a conserved amino acid within the gene encoding the catalytic subunit of the cone photoreceptor phosphodiesterase, *PDE6C*. The commonly used prediction tools classified the *PDE6C*-c.2141T>A variant to be putatively damaging/deleterious. There is only one *PDE6C*-c.2141A allele found in the Non-Finnish European population suggesting an extremely low frequency for this change. Segregation study is in progress.

Conclusions: We have identified a family affected by autosomal-recessive dystrophy of the central photoreceptor system and found homozygous possibly pathogenic variant in *PDE6C*, mutations in which have been previously reported in patients with autosomal-recessive inherited achromatopsia and early-onset cone photoreceptor dysfunction. Grant references: DUNK01/2/2009 and Grant-D-109/03.05.20018.

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P02.38D
Burden of missense variants in hearing loss genes in sporadic Meniere disease

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Introduction: Meniere disease (MD) is a rare inner ear disorder defined by episodes of vertigo, sensorineural hearing loss (SNHL) and tinnitus. MD has been described mostly in sporadic cases, being familial cases around 10% of total observed cases. Between 20-45% of the cases have bilateral hearing loss. The aim of this study is to investigate the burden of rare variants in SNHL genes in sporadic cases of MD.

Methods: We designed a targeted-sequencing panel including SNHL genes in supporting cells and sequenced 890 Spanish MD patients. The frequency of rare variants (with a MAF inferior to 0.1) in the gene panel was compared with three independent datasets as controls for the gene burden analysis. Interaction model between rare and common variants is proposed for most relevant genes in MD cases.

Results: Patients with sporadic MD showed a significant enrichment of missense variants in SNHL genes that was not found in the controls. The list of genes includes *GJB2*, *USH1G*, *SLC26A4*, *ESRRB* and *CLDN14*. A rare synonymous variant with unknown significance was found in the *MARVELD2* gene in several unrelated patients with MD.

Conclusions: There is a burden of rare variation in certain SNHL genes in sporadic MD. Furthermore, the interaction of common and rare variants in SNHL genes may have and additive effect on MD phenotype.

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P02.39A
Burden of rare variants in *OTOF* gene in familial Meniere disease

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Introduction: Meniere disease (MD) is a rare inner ear disorder characterized by vertigo, sensorineural hearing loss (SNHL) and tinnitus. Familial aggregation is found in 10% of MD cases with autosomal dominant inheritance and several genes already described, such as *DTNA*, *FAM136A* and *SEMA3D* genes. Thus, the goal of this study is to define new candidate genes for familial MD (FMD).

Materials & Methods: We recruited 62 FMD cases to perform whole-exome sequencing. Variant calling was performed with GATK. Candidate genes were prioritized based on pathogenicity using PhenIX. Single rare variant analysis (SRVA) focused on SNHL genes was performed using MAF<0.001. Likewise, a gene burden analysis (GBA) with MAF<0.05 was performed to analyze the interaction of common and rare variants. We studied the relationship between rare and common variants which modulate gene expression.

Results: Forty-percent of FMD cases carried one novel or ultrarare likely pathogenic (CADD>15) variant in SNHL genes. Ninety-four rare variants were selected from SRVA. A total of 222 rare variants were retrieved with GBA resulting in a high enrichment of variants in *OTOF* gene (corrected p-value: $3x10^{-4}$). Four rare variants in our cohort showed a significant association with three common variants regulating the expression of *OTOF* gene.

Conclusions: There is a burden of rare variants in SNHL genes. Particularly, the interaction of rare and common variants in *OTOF* gene may play an important role in FMD.

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P02.41C
Differential expression analysis suggests lipid metabolism plays a role in North Carolina Macular Dystrophy

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Introduction: North Carolina Macular Dystrophy (NCMD) is a high penetrance autosomal dominant inherited disease. The non-coding SNPs in the linkage region on chromosome 6 fall within a DNase I hypersensitive site upstream of *PRDM13* and which regulates PRDM13 expression during development. This study aimed to understand the molecular basis of NCMD through differential expression analysis.

Methods: PSSM scanning was used to predict changes in binding of known transcription factors in retina for the non-coding variants on chromosome 6. RNA-seq from skin-derived RNA for three sibling pairs (affected/unaffected each) within a family was done for differential gene expression analysis.

Results: These variants introduce a gain of binding sites for two important retinal transcription factors (PAX6 and OTX2) upstream of PRDM13. This can potentially alter the *PRDM13* regulation in retinal tissue. RNA-seq revealed ~50 differently expressed genes between the affected and unaffected. Although we did not find a direct association of these genes to the known genes involved in macular dystrophies, GO enrichment analysis showed ~30 statistically significant processes with lipid metabolism being most significant and it was previously linked to age-related macular dystrophy.

Conclusions: The introduction of PAX6 and OTX2 binding sites upstream of *PRDM13* can potentially cause PRDM13 overexpression and may interfere with lipid metabolism and oxidative stress by increasing the amount of lipids in the retina.

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P02.43A
The most common forms of non-*GJB2*-related nonsyndromic (NSHL) and disguised syndromic (SHL) hearing impartment in Russian patients

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Introduction: Hearing loss is a common disorder, and according to the audiological screening in Russia, congenital hearing impairment affects nearly 3 in every 1000 live births. Apart from the *GJB2* gene, more than 100 genes responsible for NSHL and more than 400 genes associated with SHL are known. Currently, massive parallel sequencing (MPS) is the optimal diagnostic method for non-*GJB2*-related forms of hearing impairment.

Materials and Methods: DNA samples of 205 Russian patients with NSHL (without mutations in *GJB2* gene) and 10 patients with the incoming diagnosis of Pendred, Branchiootorenal, Usher and Alstrom syndromes were tested by using custom targeted MPS panel which includes 33 genes of NSHL and SHL. 25 DNA samples were searched for abnormal copy numbers with MLPA.

Results: Among 205 patients with NSHL, pathogenic and probably pathogenic variants have been identified in 41 cases (20%) in following genes: *STRC (10), USH2A (6), SLC26A4 (4), MYO7A (4), OTOF (4), POU3F4 (2), TECTA (2), TMPRSS3 (2), PTPRQ (1), ADGRV1 (1), TMC1 (1), LOXHD1 (1), ACTG1 (1), MYO15A (1), OTOA (1)*. Diagnostic efficiency in patients with SHL was 80% (8/10). In patients with NSHL, syndromic forms have been identified in 9% of total cases (18/205). The majority of causative mutations in *STRC* and *OTOA* were large CNVs (80% and 50%). Mutations c.11864G>A, c.2171_2174delTTTG, c.107A>C in *USH2A, STRC, SLC26A4* genes are assumed to be frequent in Russian patients.

Conclusions: The proportion of detected genetic forms of hearing loss in Russia is about 12% of all non-syndromic hearing loss.

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P02.44B
Prenatal diagnosis of Norrie disease after exome sequencing of affected proband during ongoing pregnancy

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Objectives: Hereditary ophthalmic pathology is a genetically heterogeneous group of diseases that occur either as an isolated eye disorder or as a symptom of hereditary syndromes (chromosomal and monogenic). That is why diagnostic search in some cases of ophthalmic pathology could be time- and cost-consuming. The most challenging situation could arise when prenatal diagnosis is needed during ongoing pregnancy.

Materials and Methods: A family is referred to the RCMG for affected child birth risk prognosis at 7-8 week of gestation because the previous child, six-years-old boy, has congenital aniridia, glaucoma, retinal detachment, severe psychomotor delay, lack of speech, several ophthalmic surgeries. The affected child had been previously tested for *PAX6* mutations and 11p13 copy number variation which revealed no changes.

Results: Considering ongoing pregnancy, lack of pathogenic changes and precise diagnosis in an affected boy, NGS sequencing of clinically relevant genes was performed which revealed a novel hemizygous substitution NM_000266.3(*NDP*_v001):c.385G>T, p.(Glu129*), in *NDP* gene associated with Norrie disease (OMIM#310600). Subsequent Sanger validation of the affected boy and his mother confirmed identified substitution inherited in X-linked recessive mode. Amniotic fluid testing revealed the fetus is hemizygous for the variant. Complications were developed during subsequent interruption of pregnancy driven by medical necessity.

Conclusions: Clinical polymorphism of hereditary ophthalmic pathology could severely complicate the establishment of exact diagnosis and make it time- and cost-consuming. NGS appears to be the method-of-choice in complicated cases which could substantially hasten the establishment of diagnosis and genetic risk estimation.

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P02.45C
Screening TYR gene variations in Turkish oculocutaneuse albinism patients

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Introduction: Oculocutaneous albinism (OCA) is characterised by a generalized reduction in pigmentation of hair, skin and eyes and variable ocular findings including nystagmus, reduced visual acuity and photophobia. The most common mutated gene in OCA is *TYROSINASE (TYR)*. Currently, no data available on the frequency or molecular background of Albinism in Turkey.

Materials and Methods: Forty OCA patients (23 male and 17 female, 3 pairs of siblings) with a median age 19.2 years (min: 6mo-max 70.1 years) were enrolled. Following DNA isolation, the coding regions of *TYR* genes were analysed by PCR and direct sequencing.

Results: We have detected 28 different *TYR* variations in 31(77.5%, 17 homozygous, 16 compound heterozygous and 1 heterozygous) of the OCA patients (Table1). The most frequent variants were c.815G>A, p.Trp272* and c.741C>A, p.Cyc247* with an allele frequency of 0.06. Among 28 variations five of them were novel (Table 1). The allele frequencies of two common *TYR* polymorphisms S192Y and R402Q were 34% and 14.6% respectfully.

Conclusion: *TYR* gene is commonly mutated in OCA patients in Turkey. The patient with only heterozygous *TYR* variant, addition to other 8 patients with no variation should be evaluated for other related genes. Further studies with new patients and investigation of other melanin related genes in *TYR* negative patients are ongoing.

Table 1: TYR variations and their allele frequencies detected in our patients.

Gene	cNomen	pNomen	coding effect	alle freq	Known/Novel
TYR	NM_000372.4 c.815G>A	p.Trp272*	stop gain	0,0658	rs779454147
TYR	NM_000372.4 c.741C>A	p.Cyc247*	stop gain	0,0658	rs1185485047
TYR	NM_000372.4 c.996G>A	p. Met332Ile	missense	0,0526	CM91273
TYR	NM_000372.4c.1217 C>T	p. Pro406Leu	missense	0,0526	CM910385
TYR	NM_000372.4 c.1204C>T	p.Arg402*	stop gain	0,0395	CM942074
TYR	NM_000372.4 c.1 A>G	p.Met1Val	missense	0,0395	CM941342
TYR	NM_000372.4 c.573delA	p.Gly191*	stop gain	0,0395	rs1364823765
TYR	NM_000372.4 c.613C>A	p. Pro205Thr	missense	0,0395	rs61754362
TYR	NM_000372.4 c.230G>A	p. Arg77Gln	missense	0,0263	rs61753185
TYR	NM_000372.4 c.1118C>A	p. Thr373Lys	missense	0,0263	rs617543388
TYR	NM_000372.4 c.816G>C	p. Trp272Cys	missense	0,0263	11116710
TYR	NM_000372.4 c.446A>C	p. Tyr149Ser	missense	0,0263	rs797046082
TYR	NM_000372.4 c.919_925delTCCAGAA	p. Leu140fs*	frame shift	0,0263	CM90077
TYR	NM_000372.4 c.616G>A	p. Ala206Thr	missense	0,0263	rs28940880
TYR	NM_000372.4 c.140G>A	p. Gly47asp	missense	0,0263	rs61753180
TYR	NM_000372.4 c.61C>T	p.Pro21Ser	missense	0,0263	rs61753178
TYR	NM_000372.4 c.661G>T	p.Glu221*	stop gain	0,0263	novel
TYR	NM_000372.4 c.1058G>A	p. Gly353Glu	missense	0,0132	rs1415792453
TYR	NM_000372.4 c.225T>A	p.Tyr85*	stop gain	0,0132	rs746208814
TYR	NM_000372.4 c.139G>T	p. Gly47Cys	missense	0,0132	rs796051878
TYR	NM_000372.4 c.1430G>A	p.Trp477*	stop gain	0,0132	rs748052034
TYR	NM_000372.4 c.763C>T	p.Gln255*	stop gain	0,0132	CM91283
TYR	NM_000372.4 c.715C>T	p. Arg239Trp	missense	0,0132	rs774670098
TYR	NM_000372.4 c.1193A>G	p.Glu398G	missense	0,0132	CM41866
TYR	NM_000372.4 c.1255_1256insTTG		insertion	0,0132	novel
TYR	NM_000372.4 c.1036-9_1041delTAATGAACAGGATTT		splice site	0,0132	novel
TYR	NM_000372.4 c.1036+3A>C		splice site	0,0132	novel
TYR	NM_000372.4 c.308G>C	p. Cyc103Ser	missense	0,0132	novel

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P02.46D
Copy number analysis of the *OPN1LW* and *OPN1MW* genes by MLPA

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Introduction: X-linked cone dysfunction disorders such as Blue Cone Monochromacy and Cone Dystrophy are caused by pathogenic variants and rearrangements in *OPN1LW* and *OPN1MW* which share more than 98% sequence similarity. Located in tandem, they are susceptible to meiotic mispairing, unequal homologous and nonhomologous recombination. This might lead to different copy numbers of *OPN1LW* and/or *OPN1MW* or the formation of hybrid opsin genes. Because of the frequent exchange, X chromosomes have around one to five *OPN1MW* copies, whereas they rarely have more than one *OPN1LW* copy. Using the current diagnostic tests, it remains difficult to determine the copy number and presence of gene rearrangements in *OPN1LW* and *OPN1MW*.

Methods: A novel multiplex ligation-dependent probe amplification (MLPA) assay was developed and tested on 14 patient samples with a known copy number status of *OPN1LW* and *OPN1MW*. Copy number and rearrangements in *OPN1LW* and *OPN1MW* were analysed in more than 100 DNA samples.

Results: The copy number of *OPN1LW* and *OPN1MW* was confirmed in the 14 patients and could be easily determined in 75 male and female DNA samples and in 45 clinical samples consisting of affected individuals and carriers. Ten patients with hybrid opsin genes were identified, including one patient with two hybrid genes.

Conclusion: The newly developed MLPA assay enables determination of the copy number status and identification of gene rearrangements in *OPN1LW* and *OPN1MW*. The addition of MLPA to routine sequencing of *OPN1LW* and *OPN1MW* will improve detection of the cause of X-linked cone dysfunction disorders.

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P02.47A
Novel mutations in *TMEM126A* causing non-syndromic autosomal recessive optic atrophy

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Reports on autosomal recessive optic atrophy (arOA) are sparse and so far, only one gene has been specifically associated with non-syndromic arOA, namely *TMEM126A*. To date, all reports of *TMEM126A* mutations are from patients of Maghrebian origin, who all carry an identical nonsense mutation. We report two novel variants in the *TMEM126A* gene from two non-Maghreb patients/families, both resulting in an arOA phenotype.

The proband of family A was diagnosed with visual loss in early childhood but a diagnosis of optic atrophy was only made at 14 years. A diagnostic gene panel revealed a splice donor variant (c.86+2T>C) in the *TMEM126A* gene. Analysis of this variant based on RNA from whole blood revealed a single aberrant transcript lacking exon 2, presumably representing a functional null allele. Two siblings from family B were diagnosed with optic atrophy in early childhood. A missense variant (p.S36L) in the *TMEM126A* gene was identified in a gene panel-based diagnostic setting in both siblings. This missense variant is ultra rare in the general population, affects a highly evolutionarily conserved amino acid and segregates with the disease within the family. The three probands reported in this study had a relatively mild clinical course without any evidence of a syndromic (e.g. neurological) comorbidity, which is in line with previous studies.

We provide additional evidence for the implication of biallelic *TMEM126A* mutations in arOA. Our findings extend both the mutational spectrum and geographic presence of *TMEM126A* in arOA.

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P02.48B
A familial optic atrophy associated with mild neurodevelopmental disorder caused by SOX5 haploinsufficiency

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Introduction: Inherited optic neuropathies (ION) are a clinically and genetically heterogeneous group of disorders caused by nuclear or mitochondrial DNA variants. Isolated and syndromic forms are described. The most frequent ION are autosomal dominant optic atrophy (ADOA), due to *OPA1* variant, and Leber hereditary optic neuropathy (LHON), caused by mitochondrial DNA variant.

Materials and Methods: Herein we report on three patients from a family suffering from bilateral optic neuropathy and suggesting an autosomal dominant inheritance. Two of them show additional features such as speech delay, mild learning disabilities and behavior disorders. The sequencing of a panel of ION's genes including the *OPA1* gene is normal. The SNP array analysis reveals an 82 kb intragenic heterozygous deletion of the *SOX5* gene that segregates with the disease.

Discussion: The SOX5 haploinsufficiency leads to Lamb-Shaffer syndrome classically associating global developmental delay, with predominant speech impairment, mild to

severe intellectual disability and mild dysmorphic features. Some patients have additional features such as brain abnormalities or musculoskeletal anomalies. Optic atrophy is described in only two patients carrying *SOX5* heterozygous *de novo* variants: a 53 kb intragenic deletion and a point variant resulting in a premature stop codon. These two patients displayed a moderate to severe neurodevelopmental disorder.

Conclusion: Here we report on the first family of inherited autosomal dominant optic atrophy due to *SOX5* heterozygous deletion associated with mild neurodevelopmental features. This observation suggests that a *SOX5* gene analysis could be performed in patients presenting with optic atrophy associated with developmental disorder even mild.

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P02.49C
PAX6 non-coding sequence variants cause congenital aniridia

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Introduction: Congenital aniridia (AN) is a rare autosomal dominant panocular disorder caused by mutations in the *PAX6* gene. A previously conducted molecular genetic study of a large cohort of Russian patients with AN revealed several groups of *PAX6* nucleotide variants which pathogenicity is not obvious, including variants out of canonical splicing site dinucleotides, possibly affecting splicing, and 5′-UTR variants. These variants were classified as VUS or likely pathogenic according to the ACMG recommendations. Thus, to validate the pathogenicity of such variants functional studies are required.

Materials and Methods: To determine the effect of *PAX6* variants on splicing we used a minigene assay. To study 5′-UTR variants we generated luciferase constructs with full-length *PAX6* 5′-UTR (wt and mutants). The translation efficiency was measured by luciferase assay, and the RNA expression level was evaluated by qPCR.

Results: Using a minigene assay, seven out of eight investigated probably affecting slicing variants (six deep intronic and two exonic) were showed to disrupt normal splicing patterns and result in frame shifting followed by mRNA degradation by NMD mechanism. Besides, five 5′-UTR variants were found to lead to a significant decrease in the translation efficiency, including three of them, which also lead to aberrant splicing. Our further analysis allows us to suggest the mechanism of 5′-UTR variants pathogenicity through disruption of upstream ORF which possibly exists in *PAX6* 5′-UTR.

Conclusions: Using functional analysis we have confirmed the pathogenicity of 12 *PAX6* noncoding mutations. Moreover, our results suggest the exact mechanism of their pathogenic action.

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P02.50D
Mutations in *PLS1*, encoding fimbrin, cause autosomal dominant non-syndromic hearing loss (ADNSHL)

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Introduction: NSHL is a common sensory disorder characterized by high genetic heterogeneity. Despite huge efforts in genes discovery, almost half of patients still fail to receive a molecular diagnosis.

Methods: Two unrelated ADNSHL-families of European ancestry negative for mutations in known deafness-genes underwent next-generation sequencing. Data were filtered according to frequency, pathogenicity and pattern of inheritance. To prioritize genes a population-based analysis was performed (including statistics on natural selection in Europeans and gene constrains from gnomAD database) followed by *in silico* protein modelling.

Results: Among the genes found mutated in the two analysed families, interestingly, *PLS1* showed signatures of natural selection and low observed/expected ratio of both missense and loss of function mutations. Thus, these findings suggested a level of conservation and allowed to prioritize *PLS1* in which two novel likely-pathogenic missense variants were identified. *PLS1* encodes fimbrin, one of the most abundant actin-bundling proteins of the stereocilia.

Recently, it was demonstrated that *Pls1*$^{-/-}$ mice show a moderate/progressive form of HL across all frequencies (PMID: 25124451). *In silico* protein modelling displayed that both variants affect the actin-binding domain-1, and suggested an overall destabilization of the protein structure, reducing the protein's ability to bind F-actin.

Conclusion: Present results (genomic data of two independent ADNSHL-families, literature updates, evidence of evolutionary constrains and protein modelling results) provide evidence that *PLS1* is required for normal hearing and once mutated might cause ADNSHL. The identification of *PLS1* as a new ADNSHL-gene provides a new possible target for the development of therapeutic approaches.

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P02.51A
Coincidental occurrence of Schnyder corneal dystrophy and posterior polymorphous corneal dystrophy type 3

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Purpose: To report the simultaneous occurrence of two rare corneal dystrophies.

Methods: Case report of a 30-year-old male with a family history of posterior polymorphous corneal dystrophy type 3 (PPCD3) was invited for ophthalmic examination. Sanger sequencing of the coding regions and intron/exon boundaries of disease-associated genes, *ZEB1* and *UBIAD1* was performed.

Results: The clinical findings suggested co-occurrence of PPCD3 and Schnyder corneal dystrophy (SCD) in the proband. This dual diagnosis was supported by genetic findings. He was identified to carry a previously reported heterozygous nonsense mutation in *ZEB1*; c.2157C>G, p.(Tyr719*), and a novel heterozygous missense mutation in *UBIAD1*; c.569T>C; p.(Ile190Thr). The mother of the proband only carried the c.2157C>G *ZEB1* variant and slit-lamp examination of her corneas showed endothelial lesions characteristic of PPCD3. The sister of the proband carried the c.569T>C in *UBIAD1* and had corneal crystal deposition in her anterior stroma consistent with the diagnosis of SCD.

Conclusion: This case illustrates the coincidental occurrence of two rare and genetically distinct corneal dystrophies in a single patient. Furthermore, it highlights the need to perform comprehensive phenotyping in combination with appropriate genetic diagnostic testing to achieve an accurate diagnosis. This work was supported by GACR 17-12355S.

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P02.52B
Molecular genetic study of primary congenital glaucoma: the mutational analysis of *CYP1B1*and *LTBP2* genes

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Introduction: Purpose of our study was to examine the mutations of *CYP1B1* and *LTBP2* genes in patients and their relatives with primary congenital glaucoma, (PCG) and to evaluate genotype-phenotype correlations.

Patients and Methods: molecular analysis of 26 children of 21 families with PCG was performed. First we examined the common mutation p.Glu387Lys of *CYP1B1* gene using RFLP technique. In cases where wild-type or heterozygous p.Glu387Lys variant was detected, Sanger-sequencing of *CYP1B1* was performed. Common p.Arg299Ter (c.895C>T) mutation in *LTBP2* gene was also genotyped. We carried out a retrospective evaluation, using clinical data as follows: age at diagnosis, ophthalmological status, pre- and postoperative visual acuity and intraocular pressure and pedigree.

Results: we identified two mutations in 20 patients (76,92%) in our study group. The p.Arg299Ter (c.895C>T) mutation in *LTBP2* gene was found in one family in homozygous form. We identified the homozygous p. Glu387Lys mutation of *CYP1B1* gene in 12 members of 8 families, in two siblings of one family we found two deletions causing frameshift (p.Arg355Hisfs*69/p. His401Leufs*24), compound heterozygous mutations described in the literature before were identified in further 5 families. No causative mutation was detected in 6 families (28,57%) neither in *CYP1B1* gene, nor in examined position of *LTBP2* gene.

Conclusion: No genotype-phenotype correlations were found in our study group with PCG, one could not conclude on the grounds of genotype of clinical course and prognosis. This is the first study investigating the mutations of

CYP1B1 gene and the p.Arg299Ter (c.895C>T) in *LTBP2* gene of PCG in Hungary.

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P02.53C

Expanding the CPAMD8-associated eye disease spectrum to primary congenital glaucoma: lessons learned from a large consanguineous family with pseudodominance

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Primary congenital glaucoma (PCG) is defined as an isolated trabeculodysgenesis that occurs in the first three years of life and is most often caused by biallelic mutations in CYP1B1. Other early-onset, developmental glaucomas may arise secondary to developmental malformations of the anterior segment of the eye, and occur as part of an anterior segment dysgenesis (ASD) disorder. Consequently, these overlapping phenotypes may challenge the diagnosis of PCG.

Here we studied a highly consanguineous Jordanian pedigree with over 17 affected individuals in three generations, displaying a clinical diagnosis of PCG. In addition to PCG, the proband also presented with iris atrophy, ectropion uveae and corectopia. Moreover, the familial history revealed the presence of other ocular manifestations in the family including cataracts, lens luxation and iris abnormalities. Using homozygosity-based filtering of exome data, we identified a homozygous frameshift variant in CPAMD8: c.785_788del, p.Tyr262Serfs*17. This variant is not present in population databases and segregates with the phenotype in the family. Recently, CPAMD8 was reported as the cause for a unique autosomal recessive ASD that is characterized by bilateral iris hypoplasia, ectopia lentis, corectopia, ectropion uveae, and cataracts, clinical features also observed here. Interestingly, two affected siblings who were initially diagnosed with PCG received a clinical diagnosis of megalocornea in retrospect. In conclusion, we identified the molecular cause underlying a PCG phenotype in a large consanguineous family using combined homozygosity-mapping and WES. Based on this family and the previously reported families, CPAMD8-associated ASD should be taken into consideration as a differential diagnosis for PCG.

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P02.54D

Whole exome sequencing and linkage analysis of extended pedigrees to identify glaucoma susceptibility genes

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The use of next generation sequencing in extended pedigrees has significant potential for identifying functional variants linked with complex disease. We are using whole exome sequencing (WES) of five large, complex families from Tasmania (Australia) and Oregon (USA) to identify susceptibility genes for primary open-angle glaucoma (POAG), the leading cause of irreversible blindness worldwide. Extended pedigrees, enriched for POAG, provide a powerful tool to search for rare and private genetic variants influencing the disease, where enrichment of rare variants occurs as a function of segregation from the founders. The families in this study range in size from 48 to 201 individuals (28 to 91 sequenced) and span 5 to 7 generations. These families are being used to locate quantitative trait loci (QTLs) for intraocular pressure (IOP), an important glaucoma endophenotype. Variance components linkage analysis of IOP was conducted on the WES data from 249 individuals and we identified QTLs on chromosomes 2,3,6,7 and 15. The chromosome 2 locus (2q22.2-24.3) spans a 20 million base pair region. Three of the five families independently contribute linkage information to this peak, which is a novel locus for both IOP and POAG. Further analysis is being undertaken to identify genes with family specific, potentially deleterious variants; which will be validated in large POAG case/control cohorts. Finding genes involved with POAG susceptibility will increase our understanding of the biological pathways involved with the disease process and from that, diagnostic tools and more effective treatments can be developed.

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P02.55A

PRPS1loss-of-function variants, from isolated hearing loss to severe congenital encephalopathy

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We describe two sporadic and two familial cases with loss-of-function variations in *PRPS1*, which is located on the X chromosome and encodes phosphoribosyl pyrophosphate synthetase 1 (PRS-1). We illustrate the clinical variability associated with decreased PRS-1 activity, ranging from mild isolated hearing loss to severe encephalopathy. One of the variants we identified has already been reported with a phenotype similar to our patient's, whereas the other three were unknown. The clinical and biochemical information we provide will hopefully contribute to gain insight into the correlation between genotype and phenotype in this rare condition, in females as well as in males. Moreover, our observation of a new family in which hemizygous males display hearing loss without any neurological or ophthalmological symptoms prompts us to suggest analysing *PRPS1*in cases of isolated hearing loss.

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P02.56B

Biallelic sequence and structural variants in *RAX2* are a novel cause for autosomal recessive inherited rod-dominated retinal disease

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RAX2 encodes a homeobox-containing transcription factor, in which four monoallelic pathogenic variants have been previously described in autosomal dominant cone-dominated retinal disease. Here, exome sequencing in a European cohort with inherited retinal disease (IRD) (n=2086) revealed biallelic *RAX2* sequence and structural variants in five unrelated European index cases, displaying non-syndromic autosomal recessive retinitis pigmentosa (ARRP) with an age of onset ranging from childhood to the mid 40s (average mid 30s). Protein structure modeling of the novel recessive missense variants points to loss-of-function while a dominant-negative effect is predicted for the previously reported dominant *RAX2* alleles. Structural variants were fine-mapped to disentangle their underlying mechanisms and haplotyping of c.335dup in two cases suggests a common Belgian ancestry. One additional unrelated Belgian ARRP patient carried the same c.335dup allele in the absence of a second *RAX2* coding or structural variant, suggesting a role for non-coding variants in *RAX2* associated ARRP. To conclude, we found biallelic pathogenic variants in *RAX2* to be associated with ARRP, revealing *RAX2* as a novel gene for recessively inherited rod-dominated retinal diseases. The identification of *RAX2* biallelic pathogenic variants in five families of European origin indicates that this gene may underlie a non-negligible fraction of ARRP cases of other populations that still lack a molecular diagnosis. The *RAX2* mutational spectrum was broadened from sequence to structural variants (SVs). Finally, the identification of pathogenic structural variants in *RAX2* stresses the importance of SV assessment in WES and WGS data in IRD.

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P02.57C
Investigation of the role of a homozygous mutation in *BBS10* in non syndromic retinal degeneration

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Introduction: Bardet-Biedl syndrome (BBS) is rare autosomal recessive disorder, characterized by retinal degeneration, obesity, digital anomalies, genito-urinary defects, and variable cognitive impairment. It is part of a broader pleotropic class of diseases called ciliopathies. Ciliopathies are caused by mutations in genes that play important roles in the function of cellular signaling organelles called cilia. *BBS10* encodes a chaperonin–like protein that mediates assembly of the BBSome, which transports vesicles to cilia. *BBS10* is one of the most frequently mutated BBS genes, accounting for approximately 16% of cases.

Methods: A 29 yo female from a consanguineous family had non-syndromic cone-rod dystrophy (CRD) and did not have any mutation identified using standard of care clinical genetic testing. Whole exome sequencing was performed. Candidate variants were assessed based on family segregation, predicted effect on protein structure and function, amino acid conservation, and population frequency.

Results: Whole genome sequencing revealed a novel homozygous mutation in *BBS10*. The variant is rare and segregated in the family. It occurs at a residue which is highly conserved among chaperonins. Based on modelling of BBS10 secondary structure, it is predicted that the variant could 1) affect multimer assembly or 2) alter how ATP hydrolysis induces the protein conformation changes that are vital to chaperone function.

Conclusions: We have identified a rare homozygous variant we presume significant in *BBS10* in a patient with non-syndromic CRD. We are exploring the functional consequences of the variant through phenotyping a patient-derived fibroblast cell line.

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P02.58D
Clinical utility of a custom NGS panel in routine evaluation of retinal dystrophies

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Introduction: Retinal dystrophies (RD) are a broad group of clinically and genetically heterogeneous disorders affecting the retina. The non-syndromic forms of RD can be attributed to mutations in more than 100 genes. Consequently, next generation sequencing (NGS) technologies are among the most promising approaches to identify mutations in RD patients.

Materials and Methods: A cohort of patients including independent cases with various forms of RD, especially Retinitis Pigmentosa and Stargardt disease were analysed. To identify the RD causative gene, a custom *SureSelect* capture panel (Agilent) targeting 104 genes associated with non-syndromic retinal disease was developed. NGS libraries were run on Illumina MiSeq or HiSeq Sequencer. Analyses, annotation, filtration and variant curation were done using GeneSystems software (Sistemas Genómicos). When it was possible, segregation analysis of the candidate variant was performed in additional family members by Sanger sequencing.

Results: A pathogenic variant was identified in 25 RD patients (58%), including novel mutations in *ABCA4, CACNA1F, EYS* and *PRCD* gene. In 7 patients, a variant of unknown significance was detected; a functional test would be necessary to assess their clinical significance.

Conclusions: Five novel variants have been identified as a causative mutation for RD using the custom capture panel. The designed NGS assay achieves a detection rate of almost 60% and provides an adequate routine assay for genetic analysis of patients with retinal disease.

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P02.59A

Copy number variants (CNVs) identified by comprehensive genetic testing of inherited retinal dystrophies

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Retinal dystrophies (RD) are disorders that damage the photoreceptors in the retina and cause visual impairment. Prompt, comprehensive genetic diagnosis of RD can assist in risk assessment, symptom management and selection of the appropriate targeted treatment. The genetic testing needs to take into account sequence alterations and copy number variants (CNVs). We evaluated the rates and characteristics of CNVs in 2754 patients tested using a comprehensive RD panel.

DNA from patients was sequenced by targeted OS-Seq using the Illumina NextSeq500 sequencing platform or the IDT xGEN Exome Research Panel using the Illumina NovaSeq platform. CNVs were detected by CNVkit and an in-house developed deletion caller.

CNVs in 47 genes matching the patient's phenotype were reported in 4.6% of the cases. Of the CNVs, 71.1% were partial gene deletions, 13.3% whole gene deletions, 3.1% one exon deletions, and 0.8% partial exon deletions. 11.7% of the identified CNVs were duplications (partial or whole gene). 88.3% of CNVs were likely pathogenic or pathogenic, 11.7% were variants of uncertain significance. Of the likely pathogenic and pathogenic CNVs, 73.4% were diagnostic. *USH2A* and *PRPF31* were enriched in CNVs compared to other genes. Notably, CNVs were identified also in genes in which CNVs are not commonly reported, e.g. *ABCA4* and *RPE65*.

These results highlight the importance of comprehensive genetic testing for the diagnosis of retinal dystrophies. We identified CNVs ranging from one exon to whole gene deletions in multiple genes. In addition, we detected a relative high percentage of copy number duplications that warrant further investigation.

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P02.60B
Prevalence and characteristics of RPGR ORF15 variants in patients with inherited retinal dystrophies

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Introduction: The exon ORF15 in *RPGR* is a mutational hotspot for X-linked retinitis pigmentosa (XLRP). However, it generally performs poorly in standard sequencing-based assays due to a highly repetitive purine-rich sequence. To address the clinical importance of *RPGR* ORF15 and the lack of high-quality next generation sequencing (NGS) -based diagnostics, we aimed to develop a comprehensive clinical test for inherited retinal dystrophies.

Materials and Methods: We optimized a whole exome sequencing workflow with the Illumina NovaSeq 6000 platform to cover 266 retinal dystrophy-associated genes, including the difficult-to-sequence region in *RPGR* ORF15. We evaluated the performance of *RPGR* sequencing in 1587 unselected patient samples.

Results: In our clinical cohort, the overall diagnostic yield was 58%. A molecular diagnosis in *RPGR* was identified in 5.7% (90/1587) of the patients. The 90 pathogenic/likely pathogenic variants consisted of 63 frameshift (70.0%), 21 nonsense (23.3%), three missense (3.3%), and two consensus splice site (2.2%) variants, and one gross deletion (1.1%). Seventy-one out of 90 (79%) pathogenic/likely pathogenic variants were detected in the ORF15, of which 28 (39%) were in the difficult-to-sequence central region between residues p.824 and p.1077. Female patients accounted for 24% of the diagnostic cases.

Conclusions: Our results highlight the importance of *RPGR* ORF15 sequencing in retinal dystrophy patients. The high-quality NGS-based assay enables rapid and reliable molecular diagnostics of *RPGR* ORF15, and enhances the identification of patients for ongoing gene therapy trials.

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P02.61C
Prevalence and genetic characteristics of *RPE65*-associated retinal disease

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RPE65 variants are associated with severe retinal diseases including Leber congenital amaurosis (LCA) and retinitis pigmentosa (RP). Recent advancements in targeted therapy have incentivised genetic diagnostics and increased the efforts to identify *RPE65* patients eligible for therapy. We evaluated the prevalence of *RPE65* variants in 2240 retinal dystrophy patients by using next-generation sequencing (NGS).

Methods: Patients with LCA, RP, rod-cone dystrophy, or early onset retinal dystrophy were tested at a CLIA certified laboratory 2016 - 2018. Analysis was done using in-house developed and validated NGS, bioinformatics and clinical interpretation.

Results: Of 18 patients (0.8%) with *RPE65*-related disease, 7 (38.8%) had LCA, 4 (22.2%) had RP, 6 (33.3%) were affected with severe early onset retinal dystrophy, and one (5.5%) had congenital stationary night blindness (CSNB). The median age at molecular diagnosis was 13 years (range 1 - 60 years). Of the 35 disease-associated *RPE65* variants 18 (51%) were missense, 10 (29%) protein truncating, 5 (13%) splice site variants, and 2 (5.7%) copy number variants (CNVs). Two (11.1%) patients carried a CNV: a single exon deletion and a deletion of the whole *RPE65* gene, respectively. Twelve (67%) patients had at least one loss-of-function variant. One patient had the c.1430A>G, p.(Asp477Gly) variant associated with autosomal dominant disease and displayed an atypical form of RP.

Conclusions: *RPE65* has a significant role in LCA and is important in differential diagnostics of retinal dystrophies. Our results also highlight the importance of high-quality genetic diagnostics covering both sequence variants and CNVs for optimized diagnosis and clinical care.

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P02.62D

Identification of pathogenic mutations in genes involved in non syndromic hearing loss and Usher syndrome

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Non-syndromic hearing loss is characterized by a vast genetic heterogeneity; some syndromic forms have onset as isolated deafness and then evolve later in life, as Usher syndrome. We developed an NGS targeted gene-panel of 59 genes using the Ion Torrent PGM™ platform combined with a customized bioinformatic pipeline for the analysis of DNA samples from clinically highly selected subjects with sensorineural hearing loss, negative for *GJB2* mutations/*GJB6* deletions. Among the 158 subjects tested, 64 were found to carry pathogenic variants (41%) that in 18 cases (28%) altered genes involved both in NSHL and Usher syndrome (*ADGRV1, CDH23, MYO7A, PCDH15, USH1C, USH2A*); 13 of these subjects were under 15 years of age and were largely referred for NSHL. 7/18 positive-subjects carried mutation in *CDH23*, the most frequently mutated gene in our cohort. We achieved a diagnosis of Usher syndrome type I in three subjects (3 y.o.; 6 y.o.; 16 y.o.) with congenital profound hearing loss, retinal anomalies/retinitis pigmentosa and/or history of motor delay, who carried mutations in *CDH23, MYO7A* and *PCDH15*. Usher syndrome type II was diagnosed in a 12 y.o. boy referred for congenital bilateral mild hearing loss and subsequently found to have early signs of retinal alteration. 13 novel likely pathogenic mutations were identified in NSHL/Usher genes; 1 splice-site mutation has been further characterized at the RNA level. We demonstrate the importance and efficacy of integrating the powerful NGS technology with a comprehensive careful clinical evaluation, to reach an earlier diagnosis and provide important prognostic and follow-up information.

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

P03.01A
Visceral myopathy due to a novel deletion of the *ACTG2* gene: a case report

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Introduction: *ACTG2*-related disorders are a part of visceral myopathy, a rare hereditary myopathic degeneration of gastrointestinal and urinary tracts causing chronic intestinal pseudo-obstruction. Visceral myopathy is characterized by impaired intestinal function and motility resulting in severe abdominal pain, malnutrition and even death. Inter- and intrafamilial variability is present. Diagnostic criteria include absence of mechanical obstruction and histological examination of intestinal biopsies.

Materials and Methods: We report a 10-year-old boy referred to Oulu university hospital in March 2016 due to pseudo-obstruction of the small intestine. Previously, persistent ductus arteriosus was closed operatively and diagnosis of functional growth hormone disturbance was placed. He had suffered from periodic abdominal pain, vomiting and slow weight gain from infancy. His clinical picture was severe and he was completely dependent on parental nutrition. Clinical suspicion of visceral myopathy was aroused.

Results: Deletion/duplication analysis of *ACTG2* identified a heterozygous likely pathogenic deletion encompassing the entire *ACTG2*. Chromosomal microarray showed a heterozygous microdeletion 2p13.1 of 84 kb including the exons 2-9 of *ACTG2* and entire *DGUOK*. The deletion was not identified in the parents and thus, was considered *de novo*.

Conclusions: We identified a novel heterozygous deletion of *ACTG2* explaining the patient's phenotype. Mutations in *ACTG2* cause visceral myopathy. The microdeletion also included *DGUOK*. Mutations in *DGUOK* lead to autosomal recessive deoxyguanosine kinase deficiency causing neurological symptoms and liver dysfunction. No other pathogenic mutations were identified in *DGUOK*. To the authors' knowledge, no deletions in *ACTG2* have previously been reported causing visceral myopathy.

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P03.02B
The genetic background in a group of 56 Polish patients with suspected Alport syndrome

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Introduction: Alport syndrome (AS) is a clinically and genetically heterogeneous nephropathy caused by pathogenic variants in *COL4A3-COL4A5*. While most (80%) AS cases are X-linked, approximately 15% and 5% are autosomal recessive or difficult to differentiate from thin basement membrane nephropathy (TBMN) dominant forms. Digenic inheritance among *COL4A3-COL4A5* or their concomitance with other glomerulopathy or chronic kidney disease (CKD) related genes, has been recently proposed to explain the variable phenotypic expression and incomplete penetrance frequently observed in AS patients.

Materials and Methods: NGS analysis of 55 glomerulopathy and CKD related genes was performed in a group of 56 unrelated Polish patients with suspected AS.

Results: In all patients clinical diagnosis was confirmed at the molecular level. Overall, 17 known and 32 novel, likely pathogenic alterations in *COL4A3-COL4A5* were identified. The inheritance was X-linked in 73% of cases, remaining 9% autosomal recessive and 18% were dominant AS/TBMN. Changes were randomly distributed across all *COL4A3-COL4A5* coding regions, however we revealed a recurrent *COL4A5* variant c.1871G>A in twelve patients. Additionally, four patients with this alteration had likely pathogenic variant in *COL4A3*, *HNF1B* or *MYH9*, which may modify disease's severity.

Conclusions: The results of this study broaden the genotypic spectrum of AS, which will facilitate future research on the genotype-phenotype correlations. Multiple-gene sequencing is an effective approach to obtain genetic information in AS, particularly about the mode of inheritance which is important for counselling and may help to predict the clinical course, especially for those patients with mild, non-specific or atypical phenotype.

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P03.03C
Genotype/phenotype correlations in carriers of a single autosomal *COL44A3* and *COL4A4* mutation

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Alport's syndrome is complex: 85% cases show X linked inheritance with pathogenic mutations in *COL4A5* and 15% autosomal recessive inheritance with pathogenic mutations in *COL4A3* and *COL4A4*. All affected individuals develop renal failure, usually as young adults.

Carriers of a single autosomal mutation develop Thin Basement Membrane Nephropathy (TBMN) associated with 'benign familial haematuria'. We analysed a cohort of 119 individuals (29 probands and 85 relatives identified by cascade testing) with heterozygous mutations in *COL4A3* or *COL4A4* identified by Next Generation Sequencing and assessed genotype/phenotype correlations. 70% had haematuria, 38% developed proteinuria, 21% had chronic kidney disease and 10% reached end stage renal disease. 36% of our cohort had a single mutation in *COL4A4* c.2906C>G p.(Ser969Ter), representing 18% of cases of proteinuria. 50% of these patients developed proteinuria compared with 31% of patients with other mutations. The risk of eGFR falling below 60ml/min increased from 6% to 8% in patients with the *COL4A4c.2906C>G* p.(Ser969Ter) mutation. Single *COL4A3/4* mutations are recognised susceptibility factors for developing proteinuria, hypertension and renal disease in later life, but these risks have previously not been quantified. It is recommended that all patients with a single *COL4A3/4* mutation should have annual checks of blood pressure and urine, with a low threshold for prescribing an ACE inhibitor, and this is important for those with the high-risk genotype.

In conclusion, this is the largest study ever performed of the implications of carrying a single autosomal *COL4A3/4* mutation and our findings clarify the risk of renal disease in this population.

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P03.04D
Molecular genetic analysis in patients with clinically suspected autosomal recessive polycystic kidney disease

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Introduction: Autosomal recessive polycystic kidney disease (ARPKD) is a severe form of chronic kidney disease, frequently diagnosed prenatally or in an early age.

ARPKD is primaly caused by mutations in the PKHD1 gene, nevertheless, phenotype of polycystic kidneys clinically resembling ARPKD can be caused by mutations in number of other genes, such as HNF1β, PKD1, PKD2, NPHP etc. Thus, the molecular genetic analysis can be very useful in differential diagnosis of ARPKD in patient. The results of molecular genetic analysis in eleven patients with clinically suspected ARPKD, who did harbor mutations in other genes than PKHD1, are presented.

Materials and Methods: The molecular analysis was carried out using next-generation sequencing method with enrichment capture-based and amplicon-based library preparation. The panel of approximately 80 genes associated with the formation of polycystic kidneys was analyzed.

Results: The most frequent mutations found in our group of patients were variants in the TMEM67 gene (5 patients). In three patients, mutation in PKD1 was detected (in one patient in combination with PKHD1 mutation in trans). Two patients harbored combination of two mutations: (1) mutation in PKHD1 and TMEM237, (2) PKHD1 mutation and deletion of 3 exons in the NPHP3 gene. In one patient, deletion of whole HNF1β gene was identified.

Conclusions: Because of an etiologic heterogeneity of polycystic kidney disease phenotype, the complex mutational analysis, encompassing analysis in other genes (especially TMEM67), should be used for reliable differential diagnosis. Supported by the grant projects *GAUK 1015*, *PROGRES- Q25/LF1* and *RVO VFN64165*

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P03.05A
Gastrointestinal dysfunction in autism spectrum disorder: New insights from the Foxp1+/-mouse with altered gut motility and achalasia

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Gastrointestinal (GI) dysfunction is common in individuals with autism spectrum disorder (ASD), but these symptoms are often overlooked and it is still not clear how GI dysfunction relates to the core features of ASD. FOXP1 syndrome is characterized by autistic traits, such as social deficits, language impairment, and intellectual disability. But feeding difficulties, constipation, and other GI problems were also reported. Whether these symptoms are due to primary impairment or a secondary effect of altered behaviour or side effects of psychotropic medication remains unclear.

We investigated the GI tract of patient-relevant Foxp1 +/-mice. These mice have a lower body weight than wild type animals and show altered feeding behaviour. Foxp1 was expressed in all GI sections and was reduced in Foxp1 +/-mice compared with WT. A pronounced atrophy of the tunica muscularis was detected in the oesophagus and colon, caused by reduced muscle cell proliferation. Nitric oxide-induced relaxation of the lower oesophagus sphincter was impaired and achalasia confirmed in vivo by manometry. Several Foxp1 targets previously identified by microarray analysis in the brain were significantly deregulated in the oesophagus of Foxp1+/- mice. The total gut transit was significantly prolonged. Spatiotemporal maps depicting the colonic contraction patterns revealed strongly disturbed contractility and peristalsis. Overall, our findings provide the first evidence that GI disturbances in patients with FOXP1 autism spectrum disorder may be caused by impaired gut motility and achalasia, driven by FOXP1-dependent deregulation of genes. Furthermore, this is the first report of achalasia being caused by a heterozygous gene deletion.

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P03.06B
HLA and non-HLA susceptibility genes to childhood steroid-sensitive nephrotic syndrome in the Japanese population

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Idiopathic nephrotic syndrome (INS) is the most common cause of kidney disease in children, about 80% of pediatric patients respond to steroid treatment and are classified as steroid-sensitive NS (SSNS). Previous genome-wide association studies (GWASs) have identified *HLA-DR/DQ* as the predominant risk factors of childhood SSNS. Our group reported the first GWAS for childhood SSNS in Japanese. Genome-wide significant associations were identified in

HLA-DR/DQ region and disease-associated *HLA* alleles and haplotypes were further clarified (*J Am Soc Nephrol*, 2018). To identify other loci contributing to susceptibility to childhood SSNS, especially in non-*HLA* regions, we performed an extended GWAS with a larger number of samples. Discovery stage including 897 patients with childhood-onset SSNS and 2,807 adult healthy controls was carried out in the Japanese population, genotyped using Affymetrix 'Japonica Array'. Whole-genome imputation was conducted using a phased reference panel of 2,049 healthy Japanese individuals (2KJPN panel). Quality control was performed to exclude the samples with low calling rate (<97%) and the variants with low genotyping rate (<97%), minor allele frequency (MAF) <0.5% and Handy-Weinberg equilibrium (HWE) test p-value $<1\times10^{-5}$. Association analysis was conducted using logistic regression with the adjustment of gender and principal components. In this discovery GWAS, the most significant association was detected in *HLA-DR/DQ* region as we reported before ($P=2.98\times10^{-32}$, odds ratio (OR)=0.34). Furthermore, two regions on chromosome 19 and 18 showed genome-wide significant associations ($p=3.28\times10^{-18}$, OR=1.95; $p=5.38\times10^{-9}$, OR=1.65). Replication studies were successfully performed in other Asian sample sets.

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P03.07C

Two genetic disease in one family: Cockaine Syndrome (*ERCC8*) and Fanconi Bickels (*SLC2A2*)

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We report a rare consanguinous family suffering from 2 congenital autosomic recessive genetic disease. The parents are cousins and have 7 children. 3 boys and 1 girl show Cockaine syndrome (OMIM #216400) and 3 children (1 girl and 2 boys) show Fanconi-Bickels symptoms (OMIM #227810) linked to GLUT2 deficiency. Two boys are affected by both Cockaine and GLUT2 deficiency.

Fanconi-Bickels syndrome is related to *SLC2A2* mutations (3q26) and glucose transporter protein-2 (GLUT2) defect. The typical clinical picture is characterized by hepatorenal glycogen accumulation resulting in hepato- and nephromegaly. In this family the patients with SLC2A2 mutation show growth retardation, major hepatomegaly and renal failure. The homozygous mutation is intronic: lVS9-1 g>a /IVS9-1g>a.

In Cockaine syndrome, 2 genes are affected *ERCC6* and *ERCC8*. Here a new homozygous pathogenic variation has been identified in *ERCC8* (5q12.1): c.730C>T (exon 9) (Calmel N *et al. Orphanet J Rare Dis. 2016*). *ERCC8* encodes the CSA protein involved in DNA repair. The patients aging 15 to 28 years in the family show neonatal photosensibility, growth retardation, dysmorphic features, lypoatrophy, intellectual deficiency, late onset deafness and tremor. The RRS test (Recovery RNA synthesis after DNA damage) was altered and UDS test (unscheduled DNA synthesis) was normal. ERCC8 is involved in Cockaine Syndrome and UV-Sensitive syndrome, both very rare in Europe (1/200000 and 1/1000000 respectively).

The occurrence of several genetic pathology in the same family is now more frequently presented. We report here the association of two very rare syndromes affecting most children in a unique family.

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P03.08D

Apparently isolated congenital hyperinsulinism due to *KDM6A mosaic pathogenic variant*

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Introduction: Kabuki Syndrome- KS is a syndrome with multiple congenital anomalies. It is characterized by dysmorphic facial features, craniofacial and skeletal anomalies, dermatoglyphic abnormalities, mild to moderate cognitive decline, and postnatal growth deficiency. Congenital hyperinsulinism as the presenting feature of KS, was described in 10 affected individuals. The gene *KDM6A* is responsible for KS in approximately 5% of patients.

KDM6A mosaicism was reported in at least one patient with typical KS manifestations.

Case report: A 20-month-old girl diagnosed with isolated, persistent congenital hyperinsulinism responsive only to Diazoxide treatment. She has no dysmorphic features, normal development, and no structural anomalies. Molecular Analysis of the coding regions and exon/intron boundaries of 16 genes responsible for congenital hyperinsulinism was performed on a clinical basis by targeted next generation sequencing. A mosaic pathogenic variant in the gene *KDM6A* c.514C>T p.Arg172Ter was detected in at least 20%. of the cells. No other variants were identified.

Discussion: It has been suggested previously that female patients with *KDM6A* mutations have milder phenotypes than males. This is the first report of *KDM6A* pathogenic variant causing isolated congenital hyperinsulinism in an apparently healthy girl. It is likely that the combination of a mosaic state of variant and female gender resulted in congenital hyperinsulinism. We suggest the gene *KDM6A will be included in the molecular studies of isolated congenital hyperinsulinism.*

M. Yacobi Bach: None. **E. Elkon Tamir:** None. **S. Ben Shachar:** None. **O. Eyal:** None.

P03.10B
Genetic Drivers of Congenital Chylothoraces

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Introduction: Congenital chylothoraces (CCT) are rare fetal conditions, occurring in 1/10,000 pregnancies. Its clinical course varies from small thoracic effusions to life-threatening thoracic compression and secondary hydrops fetalis. Primary CCT result from malformations of the thoracic lymphoid system, secondary CCT result from cardiac anomalies or inflammatory disease. Previous studies suggest underlying genetic causes and found several genes to be mutated in some patients with lymphatic disorders. The aim of this study is to investigate new genetic drivers for primary CCT.

Methods: We applied whole-exome sequencing (WES) in two affected sibling pairs and their healthy parents. To identify disease causing variants we applied standardized filtering of WES-data. First, we filtered for autosomal-recessive and autosomal-dominant novel variants. Second, the predicted deleteriousness and the conservation of the mutations was controlled. Third, further research on the candidate genes was done to specify the functional effects of the mutations. The variants found in potential candidate genes were validated and segregated by re-sequencing. After prioritisation of the best candidate gene, re-sequencing of this candidate gene in a cohort of 30 sporadic cases is warranted.

Results: The filtering of WES-data identified four rare homozygous variants in *ZNF512* (p.His90Arg), *SMC6* (p.Thr679Met), *BRE* (p.Ser11Cys) and *GCKR* (p.Arg149Lys).

Conclusions: We suggest novel recessive candidate genes for congenital chylothoraces. Re-sequencing in a cohort of 30 sporadic cases with congenital chylothoraces and functional studies in zebrafish of the most promising candidate genes is pending.

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P03.11C
A novel homozygous nonsense mutation (p.R516X) in the *SLC5A5* gene causing congenital hypothyroidism

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Congenital hypothyroidism (CH) is the most common neonatal endocrine disorder with an incidence of 1 in 3500 live births and 2% of CH cases have familial origin. Unless a timely treatment is introduced, CH causes mental retardation and growth delay. More than 10 causative genes have been described for the pathogenesis of inherited CH to date. In this study we investigated a Mendelian cause of CH in a consanguineous family with 2 affected children. Both cases showed short stature, mental retardation and bone age retardation. Next generation sequencing analysis of 16 candidate CH genes identified a homozygous nonsense change (p.R516*, c.1546C>T) in the S*LC5A5* gene (NM_000453.2) in both cases. We confirmed the presence of this variant by Sanger sequencing and both parents and the unaffected sibling carried this variant at heterozygous state, underlying the co-segregation of the variant with the disease status in the family. Moreover, this variant was not present in 400 ethnically matched control chromosomes. p.R516* variant in the S*LC5A5* gene is not currently listed as a mutation in clinical databases. Since (i) it introduces a premature stop codon in the gene; (ii) co-segregates with the disease status in the family and (iii) is not present in 400 ethnically matched control chromosomes, here we report it as a novel mutation causing congenital hypothyroidism.

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P03.12D

Molecular analysis of *CYP21A2* gene in 100 patients with Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency

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Introduction: Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is an autosomal recessive condition in which mutations of the cytochrome P450 21-hydroxylase gene (*CYP21A2*) result in decreased synthesis of glucocorticoids and often mineralocorticoids. The clinical spectrum of the disease ranges from most severe, to mild forms depending on the degree of 21-hydroxylase activity.

Materials and Methods: Seventy-one cases (69 referred for CAH and 2 for prenatal diagnosis), as well as 29 siblings of carriers/patients with CAH, were tested for *CYP21A2* gene mutations. Long range PCR was employed to selectively amplify the *CYP21A2* gene against its pseudogene, followed by Sanger sequencing. MLPA analysis was undertaken for samples with an indication of duplication/deletion of the gene.

Results: Thirty patients with CAH were compound heterozygotes or homozygotes, while forty were heterozygotes for *CYP21A2* gene mutations. One of the prenatal cases was compound heterozygote, while the other was simple heterozygote for *CYP21A2* gene mutations. The most common mutations detected were as follows: p.V281L (25%), p.P30L (14%), I2 Splice Site (10%), p.I172N (4%), p.Q318X (2%), p.P482S (3%), p.P453S (13%) and *13G>A 3' UTR (7%). Four parents with no clinical symptoms were found to be compound heterozygotes for the non-classic form of CAH. Eight samples harbored genomic rearrangements in one or both alleles of the gene.

Conclusion: Molecular analysis of the *CYP21A2* gene is essential for proper management of patients with CAH due to 21-hydroxylase deficiency, as well as for genetic counseling and prenatal diagnosis.

E. Fylaktou: None. **A. Sertedaki:** None. **E. Charmandari:** None.

P03.13A

Application of next-generation sequencing in the search of genetic causes for Taiwanese patients with syndromic disorders of sex development

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Objective: This study aimed primarily to establish a rapid and high-throughput genetic test for syndromic disorders of sex development (DSD). Meanwhile, the applicability and effectiveness of next-generation sequencing were evaluated in searching for the remaining unknown causative genes of this inherited disease.

Methods: Patients with clinically diagnosis of DSD with additional dysmorphic features were recruited. We applied the filter-based hybridization method as the main strategy for whole exome sequence (WES) enrichment. The amplified exomes were hybridized against the DNA libraries and sequenced subsequently. We finally used the integrated computing programs to call the genetic variants for DSD.

Results: We enrolled a total of 10 syndromic DSD patients. Targeted amplicons were smoothly generated with adequate capture efficiency (coverage 98% of exons and 100% >30 read depths). Among them, we found 2 patients compatible with CHARGE syndrome (one is c.1480C>T, p.Arg494X and the other is c.6571G>A, p.Glu2191Lys in CHD7 gene), one with Robinow syndrome (c.1571delCGGGTGGGGCAGCGfs in DVL1 gene), one with OPHN1 syndrome (c.1171T>A, p.Arg391Trp in OPHN1 gene), and one with FG syndrome (c.1864C>T, p.Glu622Lys in FLNA gene).

Conclusion: We identified a number of genetic variants accounting for syndromic DSD in Taiwanese population. With identification of these causative genes, it extended our current understanding of sex development and related congenital disorders. Further functional verification of these variants may be needed on the cell line models.

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P03.14B

Splice site mutation in GRHPR gene in Iranian family with ESRD and nephrocalcinosis

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Introduction: End-stage renal disease (ESRD) occurs when your kidneys clearly begin to shut down. Both genetic and epigenetic increase risk of ESRD. A three-to nine-fold greater risk of ESRD is observed in individuals with a family history of ESRD, so role of genetic study is important nowadays.

Material and Method: A 43-year-old, Iranian man were detected ESRD with nephrocalcinosis (multiple stones). According to nephrologist decision, primary hyperoxaluria was considered so that was candidate for kidney and liver transplant. So, genetic analysis was requested to approve mentioned diagnosis. Whole exome sequencing (WES) test was performed to find variants responsible for renal disorder.

His parents had consanguineous marriage. His father and two sisters suffered from nephrocalcinosis. According to results of WES test and analysis all genes responsible to renal disorder, one likely pathogenic/pathogenic variant (c.735-1G>A) in splice site region was found in *GRHPR* gene. This gene is responsible for primary hyperoxaluria type 2 with an autosomal recessive inheritance pattern.

Clinical manifestations in patients were same as Mentioned disease, after examining of clinical data. Familial segregation was done and his father and two sisters are carrier of this variant. Liver transplant might be considered within kidney transplant in this case.

Conclusion: Genetic analysis should be considered in renal failure to do the best management in therapy.

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P03.15C
GCK mutations in Croatian MODY patients

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Introduction: Maturity onset diabetes of the young (MODY) is clinically and genetically heterogeneous group of diabetes inherited in autosomal dominant manner. It usually occurs in adolescence or young adulthood and accounts for at least 1-3% of all diabetes. GCK-MODY is one of four most common type of MODY with estimated prevalence of 1:1000. It is characterized by mild, stable fasting hyperglycemia which is often discovered incidentally during routine medical screening.

Materials and Methods: After clinical examination, 56 patients with stable hyperglycemia, small 2 hour increment in OGTT, positive family history of type 2 or gestational diabetes and negative pancreatic antibodies were tested for GCK-MODY. The promoter, whole coding region and flanking intronic regions of the *GCK* gene were analyzed by Sanger sequencing. Pathogenicity of identified mutations was verified in reference databases for mutations related with GCK-MODY.

Results: 17 different mutations in *GCK* gene were found in 32 patients. Most of the mutations were in exon 7 (six mutations in 16 patients) and in exon 9 (five mutations in 5 patients). The most common mutation was p.Thr228Met in exon 7 found in 8 patients from 4 different famillies. One patient was apparently homozygous for mutation p. Gly170Asp, but its true homozygosity is not yet confirmed, it can be a result of an allele dropout due to SNP in the primer region. In one patient we detected a novel variant c.806T>G, p.Phe269Cys in exon 7.

Conclusions: GCK-MODY is frequently underdiagnosed and inadequately treated. Treatment is rarely necessary if the mild hyperglycemia remains stable.

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P03.16D
Novel variants in *DHH* gene identified with 46,XY gonadal dysgenesis

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Introduction: Desert Hedgehog (DHH) a member of the hedgehog family, is located in 12q13.1 and acts on early testicular development, testis cord formation and differentiation of fetal Leydig cells. To date, six homozygous mutations have been described in DHH in 46,XY patients conferring phenotypes ranging from partial to complete gonadal dysgenesis, associated with of without polyneuropathy.

Materials and Methods: We investigated three patients from two families with 46,XY gonadal dysgenesis, for pathogenic sequence alterations in 31 associated genes, with in-house-designed next generation sequencing (NGS) targeted gene panel, using an *Ion Torrent* platform. Ultrasound

and histopathological examination of the gonads with electrophysiological examination of peripheral nerves were performed. Protein modeling was done to predict the effect of the missense mutations.

Results: We have identified three different homozygous mutations, one in two siblings, c.[1146G>A];[1146G>A], (p.[Trp382*];[Trp382*]), and two in singleton case, c. [71G>C;1063C>T];[71G>C;1063C>T], (p.[Gly24Ala; Arg355Cys];[Gly24Ala;Arg355Cys]). One of the siblings presented with penoscrotal hypospadias, bilateral inguinal testes, Mullerian structure evident on biopsy, no response to HCG at one-year and raised as female initially. Second sibling presented micropenis, bilateral inguinal testes, no Mullerian structure, response was normal to HCG at age 14 days and raised as male. In addition, both patients suffered from polyneuropathy. Third patient presented at age 19 days with penoscrotal hypospadias, bilateral inguinal testes, no Mullerian structure, low AMH and raised as male.

Conclusions: *DHH* mutation should be analyzed in patients with 46,XY gonadal dysgenesis for diagnosis and the presence of potential neuropathy and gonadal tumors.

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P03.17A
HBx destabilizes TIP60 in HBV-induced hepatocellular carcinoma

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TIP60(TAT-interactive protein of 60 kDa),is a lysine acetyltransferase (KAT5) and acts as a haplo-insufficient tumor suppressor in multiple cancer-types. TIP60 is known to be destabilized by different onco-viruses and this intrigued us to inspect its role in another onco-virus pathogenesis, hepatitis B virus (HBV). HBV, the leading cause of hepatocellular carcinoma (HCC) is the 6th most common cancer in the world. Among the four overlapping open reading frames of HBV, the HBV X gene (HBx) is considered the causative agent for malignant transformation associated with HBV infection. In this conference I am going to discuss data that show TIP60 being targeted by HBx, a proto-oncogene of HBV proteasomally, by utilizing an E3 ubiquitin ligase, EDD1 in the cell. By utilizing various cell culture models, we demonstrate that HBx and TIP60 form a complex with EDD1. Interestingly, this hacking of TIP60 protein, enables increased proliferation of hepatocytes, as confirmed by various growth assays and mechanistically by increased expression of *TERT* and decreased levels of TIP60 protein. Additionally, tissue microarray analysis(TMA)of HBV-positive malignant tumor samples demonstrate a strong correlation between TIP60 and EDD1 levels, further supporting our hypothesis. We therefore propose TIP60 as one of the cellular targets in HBx mediated viral infection and to utilize small molecule inhibitors against the identified ubiquitin-ligase in HBx-mediated HBV-carcinogenesis. In conclusion, our study has identified a relatively unknown role of HBx, its destabilization of an epigenetic writer and the mechanism involved in this phenomenon. This work was supported by MOE grants (MOE AcRF Tier 1 T1-2012 Oct-04 and T1-2016 Apr-01) and CSI(R-713-006-014-271). SH is supported by NUS-Research Scholarship awarded by NUS Yong Loo Lin School of Medicine.

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P03.18B
Next generation sequencing approach for determining the molecular basis of familial hematuria

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Introduction: Hematuria is a non-specific symptom of kidney or urinary tract pathology. The presence of blood in the urine could be due to trauma, infections, systemic disease, disorders of the coagulation or genetic mutations affecting the development and function of particular cell and tissue types. The presence of family history is indicative of a hereditary condition such as disorders of the basement membrane, complement or podocytes. Differential diagnosis of hematuria is crucial for successful treatment of the underlying condition.

Materials and Methods: Illumina TruSight One Sequencing Panel was used for mutation screening in 8 families with multiple members with hematuria. The index patients were referred with initial diagnoses of glomerulopathy, thin basement membrane nephropathy, uric acid nephropathy, focal segmental glomerulosclerosis, tubulointerstitial nephritis and/or chronic kidney disease.

Results: Using next generation screening we determined the genetic cause of hematuria in Bulgarian families. Pathogenic variants were found in the type IV collagen (autosomal recessive and dominant variants in COL4A4, X-linked in COL4A5) and complement genes. Both missense and splice mutations, known pathogenic and novel variants were found. A potential role of heterozygous C1QC mutations in the pathogenesis of G3 glomerulopathy was observed.

Conclusion: Clinical diagnosis of hereditary hematuria is often hindered by phenotype variability, lack of information for disease progression in older family members, difficulties in obtaining biopsy samples, etc. Addition of massive parallel sequencing of large gene to the diagnostic procedure would allow timely and precise determination of the molecular cause of the disease.

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P03.19C

A rare diagnosis: Hermansky-Pudlak syndrome in a patient with pulmonary fibrosis, oculocutaneous albinism and thrombocytopathy

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Introduction: Hermansky-Pudlak Syndrome (HPS) is an autosomal recessive disorder associated with oculocutaneous albinism, bleeding diathesis, granulomatous colitis, and highly penetrant pulmonary fibrosis in some subtypes, Homozygous or compound heterozygous mutations in *HPS1, HPS3, HPS4* and several other genes lead to clinical manifestation of the disease.

Materials and Methods: A 57-year-old patient with congenital oculocutaneous albinism, thrombocytopathy and late onset accelerated pulmonary fibrosis was referred to our clinic. Negative family history of these symptoms suggested autosomal-recessive mode of inheritance. We performed NGS analysis of proband-parents trio. Whole-exome libraries were prepared according to the Nimblegen SeqCap EZ Exome v3 protocol and sequencing was performed on NextSeq 500 for all of them. Furthermore, we performed *in silico* analysis of a virtual gene panel, including *HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, BLOC1S3*, and *PLDN*.

Results: Whole-exome sequencing identified a compound heterozygous genotype in *HPS1* gene in the proband. We identified a pathogenic frameshift variant c.1189delC (p.Gln397Serfs*2), resulting in a premature stop codon, associated with HPS. Furthermore, we identified a rare, previously undescribed nonsense variant, c.1507C>T (p.Gln503*), resulting in a premature stop and mRNA degradation. Presence of both variants was verified by Sanger sequencing. The following molecular-genetic analysis of parents confirmed their heterozygous carrier status.

Conclusions: Compound heterozygous mutations in *HPS1* in the proband lead to disruption of *HPS1* gene and clinical manifestation of Hermansky-Pudlak syndrome with severe pulmonary fibrosis leading to respiratory failure and death. This study was supported by Czech Ministry of Health (grant AZV 16-29447A) and Masaryk University (grant MUNI/A/1105/2018).

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P03.20D

Genetic testing for hereditary hemorrhagic telangiectasia diagnosis: identification of new ENG and ACVRL1 mutations in Italian families

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Introduction: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disease characterized by mucocutaneous telangiectasias, epistaxis and visceral arteriovenous malformations (AVMs). HHT-type-1 is caused by ENG gene mutations and it is characterized by a high incidence of pulmonary and cerebral AVMs. Type-2 is caused by ACVRL1 gene mutations and it is associated with hepatic AVMs. Mutations in SMAD4 gene cause Juvenile Polyposis/HHT syndrome. Here we report two

cases with suspected HHT-1 and HHT-2 in which new ENG and ACVRL1 mutations where found.

Materials and Methods: DNA was extracted from blood samples of patients with suspected HHT. ENG, ACVRL1 and SMAD4 genes were analyzed by Next Generation Sequencing and results were confirmed by Sanger Sequencing.

Results: Case-1: mutation c.780_781insCCTACG (p. Ser260_Trp261insProTh) on ENG gene was identified in a 16-year-old boy with epistaxis, PAVM and HHT-1 family history. This mutation hasn't been reported yet. Genetic testing was also performed in patient's three brothers and two cousins. The mutation was identified in one brother (4-years-old, experiencing bleeding episodes) and both cousins (9 and 7 years-old). Two brothers (15 and 9 years-old, without any HHT symptoms) resulted negative. Case-2: mutation c.1327T>G (p.Cys443Gly) on ACVRL1 gene was identified in a 45-years-old woman with severe liver AVMs and enlisted for transplantation, rare epistaxis and no HHT family history. The mutation, consistent with clinical suspect of HHT-2, hasn't been described yet.

Conclusions: Here we report new ENG and ACVRL1 mutations thought to be causative of hereditary hemorrhagic telangiectasia. Further clinical evaluation on carrier and not-carrier relatives will be performed.

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P03.21A

Enrichment for large deletions encompassing genes expressed in the enteric nervous system in Hirschsprung disease patients with additional associated anomalies

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Hirschsprung disease (HSCR) is characterized by absence of enteric ganglia, primarily in the distal colon. Approximately 18% of patients have additional anatomical malformations or associated neurodevelopmental disorders, including autism and intellectual disability. A subset of these patients have a known genetic syndrome in which HSCR has a variable expression or penetrance. In others, the genetic etiology is unknown and we hypothesize that rare Copy Number Variation (CNV) impacts their disease development. Indeed, rare Copy Number (CN) losses were significantly enriched in patients with HSCR and additional anomalies without a known causal variant (n=23, p=3.64E^{-7}), and not in isolated HSCR (n=20, p=0.700) or in HSCR patients with a known *RET* or other causal variant (n=15, p=0.705). Of the HSCR patients with additional anomalies tested, at least five (three males and two females) had a large *de novo* CNV and one male inherited an X-linked CN loss. Patients with a known causal variant had a significant lower burden of the known HSCR predisposing risk haplotypes (P=0.0232), and isolated HSCR patients a higher burden (P=0.0277) compared to patients with HSCR and additional anomalies without a known causal variant. The rare CN losses identified are enriched for dosage sensitive genes, expressed in the developing mouse enteric nervous system (ENS; p=1.760E-10): *SLC8A1*, *DDR1*, *GNL1*, *GABBR1*, *MAPK8*, *UFD1L*, *FHIT*, *AKT3*, *TUBB*, *TBX2*, *BCAS3* and *USP32*. Additionally, the latter four, had rare putative deleterious variants in HSCR patients without a deleterious CNV, confirming our hypothesis that rare CNV contributes to syndromic HSCR with unknown genetic etiology.

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P03.22B

The impact of*NRG1*expressions and methylation on multifactorial Hirschsprung disease in Indonesia

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Background: Hirschsprung disease (HSCR) is a complex genetic disorder characterized by absence of ganglion cells in the gastrointestinal tract. Our previous study revealed that *NRG1* rare variant frequency in Indonesian HSCR patients is <1%. Therefore, we wished to investigate *NRG1* expression and methylation effect on HSCR pathogenesis in Indonesia.

Methods: *NRG1* type I (*HRGα, HRGβ1, HRGβ2, HRGβ3, HRGγ* and *NDF43* isoforms), type II and type III expressions in both ganglionic and aganglionic colon of HSCR patients and control colons were analysed by real-time polymerase chain reaction (RT-PCR). Next, we treated the extracted gDNA from HSCR patients' and control colons with sodium bisulfate and analyzed the methylation pattern of *NRG1* exon 1 with methylation-specific PCR.

Results: *NRG1* expressions were up-regulated in HSCR patients colon compared with control (type I: 13.81±1.79 vs.15.22±1.22 vs. 17.78±1.27; type II: 13.93±1.52 vs. 15.67±1.52 vs. 16.57±1.02; type III: 11.98±2.86 vs.16.24 ±2.28 vs. 17.51±1.48, respectively), with *p*-value of 0.02, 0.03 and 0.01, respectively. Furthermore, *HRGβ1/HRGβ2* expressions almost reached a significant difference between ganglionic and control colons (13.07±1.32 vs. 14.75±1.26, $p=0.09$). Most HSCR patients (80%) and controls (75%) revealed partially methylated *NRG1*. *NRG1* methylation levels were lower in the ganglionic and aganglionic than control colons, however did not reach a significant level ($p=0.31$ and 0.13, respectively).

Conclusions: We shows the aberrant *NRG1* expression in Indonesian HSCR patients and might not be due to DNA methylation. Moreover, our study provides further insights into the contribution of aberrant *NRG1* expression in the HSCR pathogenesis.

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P03.23C

The circadian aspect of *PNPLA3* p.I148M and *TM6SF2* p.E167K in hypercholesterolemia

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Background and aims: Lipid metabolism is under circadian control and long-term disturbance can lead to metabolic syndrome. Herein we test the hypothesis that variants rs738409 (p.I148M) in *PNPLA3* and rs58542926 (p.E167K) in *TM6SF2* associated with pathogenesis of Non-Alcoholic Fatty Liver Disease also represent novel hypercholesterolemia-associated variants. We propose that both non-synonymous variants associate with the change of circadian behavior in hepatic cells and contribute to the development of the hypercholesterolemia.

Materials and Methods: DNA samples from a Slovenian general screening programme of preschool children, age of 5, with known total cholesterol and LDL-cholesterol diagnosed with FH were obtained (N=121). The frequency of variants rs738409 and rs58542926 were compared to European individuals from 1000 Genomes and Exome variant server databases. With CRISPR/Cas9 technology we generated HepG2 cells with different *PNPLA3* and/or *TM6SF2* genotypes and assessed the metabolic, expression and proliferation aspects.

Results: An association between the *PNPLA3* rs738409 and *TM6SF2* rs58542926 minor allele with hypercholesterolemia has been identified if compared to European males from 1000 Genomes (OR=1.451, 95%CI=1.093-1.926, p<0.01; OR=0.3583, 95%CI=0.1536-0.8358, p<0,05) and European Americans from EVS database (OR=1.491, 95%CI=1.075-2.097, p<0.05; OR=0.3621, 95%CI=0.1603- 0.8181, p<0,01). HepG2 cells were found homozygous for rs738409 minor allele and rs58542926 major allele. We currently evaluate the circadian expression of both genes and the effect of cholesterol on genotypes of genetically modified cell lines.

Conclusions: We propose both polymorphisms as a novel hypercholesterolemia-associated genetic variant that disrupts the circadian rhythm and contribute to elevated plasma lipids.

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P03.24D

Large deletions are an underappreciated cause of hyperinsulinism

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Introduction: Hyperinsulinism is a disorder where dysregulated insulin secretion leads to hypoglycaemia. 50% of patients do not have a mutation in a known gene. Large contiguous gene deletions have been reported as an extremely rare cause of hyperinsulinism but are not routinely screened thus these may be an underappreciated cause of the disorder.

We aimed to assess the contribution of large deletions to the aetiology of hyperinsulinism.

Materials and Methods: Using off-target CNV (copy number variant) calling from targeted next generation sequencing data we screened 865 patients with hyperinsulinism for large deletions (>1Mb).

Results: We identified causative contiguous gene deletions in 16/865 patients in our cohort. 13 were previously reported to cause hyperinsulinism: X chromosome deletions (Turner syndrome) (n=3), 9p deletions (n=9) and a 16p deletion (n=1). We also identified 3 patients with overlapping *de novo* deletions on chromosome 20. These were the only novel large (>1Mb) *de novo* deletions within the cohort. They are a new cause of hyperinsulinism.

Conclusions: 2% of patients in our cohort had causative large contiguous gene deletions. This is likely to be an underestimate of the prevalence of large deletions in hyperinsulinism as some patients will have had cytogenetic testing prior to referral for hyperinsulinism genetic testing. Large deletions are a rare but significant cause of hyperinsulinism and should be screened for as part of genetic panel tests for the disease. We also highlight a novel cause of hyperinsulinism: 20p11.2 deletions.

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P03.25A
Searching for genes related to idiopathic central precocious puberty (ICPP) in a Spanish cohort

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In the last decade, the number of Idiopathic Central Precocious Puberty (ICPP) cases has decreased thanks to the discovery of mutations in different genes. The identification of loss of function of the maternal imprinted gene *MKRN3* is the principal genetic cause of ICPP[1-12]. More recently *DLK1* has also been discovered as another imprinted gene implicated in the pathogenesis of ICPP[13].

Aims of the study: 1) To analyze the presence of pathogenic variants in 13 genes related to GnRH pathway; 2) To evaluate the diagnostic yield of the coding region analysis of *MKRN3;* 3) To determine the presence of copy number variation (CNV) of *DLK1* and *MKRN3* genes; 4) To exclude uniparental disomy (UPD) of chromosome14.

Patients and Methods: patients with ICPP were gathered from 2015 to 2018. In 20 patients we analyzed the clinical exome filtered by 13 genes that included *MKRN3*. Additionally, in another 11 patients we analyzed *MKRN3* coding region by Sanger sequencing. In all patients we analyzed: a) *DLK1* coding region by Sange sequencing; b) CNVs of *MKRN3* and *DLK1* by MLPA technique; c) UPD of chromosome 14 using STRs markers.

Results: from the 31 patients recruited we found two likely pathogenic variants in *MKRN3* in one sporadic case and in one familial case.

Conclusion: *MKRN3* gene is the most frequent genetic cause both sporadic and familial ICPP so we propose it as the first one to be screened in the genetic approach of patients with ICPP.

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P03.26B
Complex indel variant calling in a repetitive genomic region

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Introduction: Interstitial Lung Disease (ILD) represents a collection of many different pulmonary disorders that interfere with the tissue and space surrounding the alveoli, causing irreversible damage. We performed genetic testing on a patient diagnosed with idiopathic pulmonary fibrosis who also had telomere lengths in the 1st centile.

Materials and Methods: Next-Generation sequencing was performed on patient DNA using our in-house Respigene™ panel of 172 genes. An in-house bioinformatics pipeline, configured to interpret genes associated with rare respiratory conditions, was used to classify SNVs and CNVs.

Results: Initial analysis did not detect any potentially pathogenic variants in genes associated with ILD in the patient. Another laboratory reported a *RTEL1* splice variant: c.1266+3A>G. Further investigation using different variant callers detected a 78bp deletion at the *RTEL1* exon 15/intron 15 boundary. Sanger sequencing and Tapestation analysis confirmed the patient to be heterozygous for the 78bp deletion. This deletion is classified as a VUS, but we consider it more likely pathogenic than benign. The c.1266+3A>G variant showed an allelic balance of less than 40%, and our results suggest it is an artefact caused by bioinformatic misalignment of the complex repeat region.

Conclusion: Complex and repetitive genomic regions still prove challenging for routinely-used variant calling software and Sanger sequencing. The error in variant calling may have resulted in a missed genetic diagnosis in this patient. Increasing intronic flanking regions for bioinformatic analysis when performing targeted sequencing and 'training' of bespoke bioinformatic pipelines will improve detection of complex variants in the exon/intron boundaries and beyond.

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P03.27C

Changes in human physiology in response to three-week inactivity or how your training trains your microbiome

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Introduction: We explored pathophysiological consequences of inactivity and hypoxia on human physiology and intestinal microbiota in healthy males during the randomized crossover design of run-in (5 day) and experimental phases [21-day normoxic bedrest (NBR), hypoxic bedrest (HBR) and hypoxic ambulation (HAmb) (hypoxic ~4000 m simulated altitude)] in a strictly controlled laboratory environment, with balanced fluid and dietary intakes and 24/7 medical surveillance.

Materials and Methods: Incorporating system medicine approach, intestinal transit spanning constipation, eosinophil-derived neurotoxin, bile acids, diet composition, intestinal electrical conductivity, indole etc., along with NMR metabolomics, were assessed. Furthermore, structure, diversity and function of intestinal microbiota using shotgun metagenomics were investigated. Finally, all observations were integrated and analyzed in correlation to negative physiological symptoms, related to obesity and metabolic syndrome in same participants.

Results: Inactivity negatively affected fecal consistency and in combination with hypoxia aggravated the state of gut inflammation. On the other hand, many of the microbial parameters were shown to lag behind the changes in human physiology and intestinal environment, since significant changes in bacterial community were delayed until week four in HBR only, where members of the genus *Bacteroides* and proteins involved in iron acquisition and metabolism, cell wall, capsule, virulence and mucin degradation were enriched.

Conclusions: Our multi-omics approach suggest a time-dependent and complex interplay between the host physiology (including apparent constipation), immunity (inflammation), controlled diet, intestinal environment variables and microbiome physiology during the acute cessation of exercise. Funding: PlanHab project (Grant no. 284438) and Young Research Fellowship to RŠ (SRA#37426).

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P03.28D

A novel homozygous *CARMIL2* variant leads to infantile-onset colitis and gastrointestinal eosinophilic disease without recurrent infections

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Introduction: Infantile-onset colitis refers to inflammatory gastrointestinal (GI) disorders manifesting before the two years of age. Nearly 100 genes have been implicated in syndromes presenting with early-onset colitis. We aimed to decipher the genetic basis of severe infantile colitis and eosinophilic GI disease without recurrent infections in a 10-years-old boy, and subsequently characterize his GI and immunological phenotypes.

Materials and Methods: We used trio whole exome sequencing (WES) for pathogenic variant discovery. Protein expression was studied using western blot (WB) analysis and immunohistochemical (IHC) staining. Immunological workup included *in vitro* T-cell studies, flow cytometry and CyTOF analyses.

Results: WES revealed a rare homozygous variant in *CARMIL2:* c.1590C>A; p.Asn530Lys. The variant was predicted pathogenic and co-segregated with the disease in the nuclear family. WB and IHC studies revealed markedly reduced CARMIL2 expression in patient cells compared to controls. Comprehensive *in vitro* immunological analyses demonstrated severely reduced regulatory T-cells (T_{reg}) with impaired $CD4^+$ T cell proliferation and T_{reg} generation. CyTOF analysis revealed significant shifts in the innate and adaptive immune cells of the patient compared to ulcerative colitis patients and healthy controls.

Conclusions: Pathogenic bi-allelic *CARMIL2* variants cause an immunodeficiency syndrome characterized by recurrent infections and skin lesions, occasionally with concurrent diarrhea. This study expands our knowledge on the immune landscape alterations caused by CARMIL2 defects and underscores the role of *CARMIL2* as a candidate gene for early-onset inflammatory and eosinophilic GI disease. The predominant GI manifestations in the patient warrant further study of CARMIL2 function in the gut.

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P03.29A

Clinical utility of gene panel sequencing for diagnosis of early-onset chronic kidney disease

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Introduction: Inherited kidney diseases (IKD) are the leading cause of chronic kidney disease (CKD) in the first three decades of life and encompass a broad range of nephropathies.

Methods: Targeted next generation sequencing with high sequencing depth of a large IKD-gene panel was performed in 426 patients with early-onset CKD (<30 years), including patients with: 1) congenital abnormalities of the kidney and urinary tract (CAKUT) (n=89) (with bilateral anomalies and/or extrarenal defects and/or familial CAKUT), 2) renal cystic ciliopathies (n=180), 3) suspected inherited glomerular disease (n=129) and 4) renal tubulopathies/nephrolithiasis/nephrocalcinosis (n=28).

Results: Causative mutations were found in 64% (271/426) of patients: 42% (37/89) with CAKUT, 76% (137/180) with renal cystic ciliopathies, 60% (77/129) with suspected inherited glomerular disease and 71% (20/28) with renal tubulopathy/nephrolithiasis/nephrocalcinosis. Copy number variants were detected in 11% of all genetically diagnosed patients (29/271). Pathogenic variants were identified in 57 different genes, 10 of which (*COL4A3, COL4A4, COL4A5, HNF1B, NPHP3, PAX2, PKD1, PKD2, PKHD1* and *TSC2*) explained 75% (203/271) of patients.

Conclusion: Our IKD-gene panel allowed a precise molecular diagnosis in nearly two-thirds of patients with early-onset CKD. The high diagnosis yield of our approach can be explained by 1) the efficient detection of copy number variants and variants in complex genomic regions, such as *PKD1* gene, due to the high sequencing depth and 2) the strict clinical inclusion criteria to favor genetic testing in patients with likely monogenic cause of nephropathy.
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P03.30B
Large effect of rare variants with large effect sizes in (sporadic) IPF

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Introduction: Rare variants in telomere metabolism associated genes have recently been shown to be important contributors to idiopathic pulmonary fibrosis (IPF), regardless of the family history. We aimed to validate the result in an independent cohort of IPF patients.

Methods: We calculated that in order to have 80% power to detect at least one IPF patient with a causative mutation in a telomere metabolism associated gene, if the prevalence of mutations is the same as in the above referenced study (13.2%), we had to sequence 11 patients for the presence of mutations in those genes. To get a random sample we included 11 consecutive IPF patients, regardless of their family history of IPF, at their control visit at our University Clinic. All patients had IPF diagnosis according to ATS/ERS/JRS/ALAT guidelines. Sequencing of telomere metabolism associated genes was performed on the Illumina platform.

Results: Demographic statistics of our sample were comparable to other IPF demographics with regard to age (mean age = 71.2 years), IPF family history (1/11 (9.1%)) and sample male predominance (8/11 (72.7%)). In 2 out of 11 (18.2%) patients, none of which had a family history of IPF and were therefore classified as sporadic IPF cases, the causative variant was found (NM_001283009(RTEL1): c.326_329del (p.I109fs); NM_001193376(TERT): c.1374delC (p.W459fs)). Despite sufficient coverage (mean coverage = 121X) we didn't confirm the causative variant in the only patient with a family history of IPF in our cohort.

Conclusions: Causative variants in telomere metabolism associated genes play a significant role also in sporadic IPF.

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P03.31C
A new form of hereditary iron overload unlinked to known hereditary hemochromatosis genes

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Hereditary hemochromatosis (HH) is normally caused by homozygosity for pathogenic mutations in the *HFE* (homeostatic iron regulator) gene. Other autosomal recessive forms of HH are associated with mutations in genes involved in iron homeostasis including hemojuvelin (*HJV*), hepcidin (*HAMP*) and transferrin receptor 2 (*TFR2*). Autosomal dominant forms of iron overload are associated with mutations in the cellular iron exporter ferroportin (*SLC40A1*), with a single case linked to a mutation in the 5'UTR of the H-ferritin gene (*FTH1*). We describe a family

with apparent autosomal dominant iron overload with characteristics similar to the classical form of ferroportin disease. The condition is characterised by elevated serum ferritin levels with normal or mildly elevated transferrin saturation. Perls' Prussian blue staining of liver biopsy sections in two affected individuals show prominent iron deposition in both Kupffer cells and hepatocytes. Sequencing of the coding sequences and splice sites of genes known to cause HH revealed no mutations in the proband. Whole genome SNP genotyping was performed on 15 members of the pedigree. Linkage analysis showed haplotypes on regions of chromosomes 1, 3, 12, 18 and 19 that were shared among the four most clearly affected members of the pedigree but not present in the three most clearly unaffected members. These regions encompass a total of 93 megabases, approximately 3% of the human genome and contain over 1500 canonical genes. Importantly, these genomic intervals do not contain the *SLC40A1* gene indicating that affected members of this family do not have a variant form of ferroportin disease.

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P03.32D

Transcriptome profiling in regenerating rat liver after partial hepatectomy, portal vein ligation, and associated liver partition and portal vein ligation for staged hepatectomy

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Introduction: Liver has phenomenal capacity to regenerate, which has been shown clinically where diseased liver parts are removed to preserve and stimulate the growth of the remaining healthy tissues. Several surgical techniques have been developed for such purpose, including partial hepatectomy (PH), intraoperative portal vein ligation (PVL), and associated liver partition and portal vein ligation for staged hepatectomy (ALPPS). Indeed, while clinical outcomes are somewhat comparable, different procedures show clinically distinct recovery patterns. The observable clinical differences likely mirror some underlying variations in the patterns of gene activation and regeneration pathways.

Materials and Methods: We provided a comprehensive comparative analysis of the gene regulation in regenerating rat livers temporally spaced at 24h and 96h after PH, PVL, and ALPPS using the next-generation RNA sequencing transcriptomics analysis.

Results: The time-dependent factors appear to be the most important determinant of post-injury alterations of gene expression in liver regeneration. Early transcriptomic changes that were found in all three procedures included cell cycle associated genes and immune-response activation genes as well as transcription factors, DNA replication regulators, G1/S-transition regulators, and cytokinesis.

Conclusions: The functional pathway and gene network analyses revealed both unique and overlapping molecular mechanisms and pathways for each surgical procedure. Identification of molecular signatures, such as gene-to-gene interactions, regenerative signaling pathways, specific to each surgical procedures further our understanding of key regulators of liver regeneration as well as patient populations that are likely to benefit from each procedure. Funding: This study is funded by KFSHRC Research Grants (2110006 and 2180030 to DC).

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P03.33A

Biallelic *LZTR1* mutation in a patient with non-classical Noonan phenotype

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Noonan syndrome (NS) is an autosomal dominant, multisystemic disorder caused by dysregulation of the RAS/

mitogen activated protein kinase (MAPK) pathway. Heterozygous variants in 11 known genes account for approximately 80% of cases. Whole exome sequencing (WES) studies recently identified the existence of a recessive form of Noonan syndrome caused by biallelic pathogenic *LZTR1* variants, however information on the phenotypes of *LZTR1* patients and functional properties of the mutations are limited. We report a 15-year-old boy with typical facial features of NS in the absence of congenital heart defects, cardiomyopathy, skin anomalies and intellectual disability. The patient had been treated with growth hormone (GH) between ages 11 and 15 without any effect on growth. WES revealed compound heterozygosity for a missense *LZTR1* variant c.2102C>G; p.Pro701Arg inherited from his healthy father and a missense variant c.2062C>G: p.Arg688Gly NM_006767 inherited from his mother. We identified the variant inherited from his mother by exome reanalyses modifying pipelines parameters. Both variants were in the BTB domain of LTZR1. Mutations in *LZTR1*, already known to be causal in familial schwannomatosis type 2, have been recently involved in some patients with autosomal dominant and recessive Noonan syndrome in which clinical manifestations include cardiomyopathy and intellectual disability. This is the first case in which only facial features, short stature and no response to GH therapy are reported. Recently *LZTR1* was associated to sporadic cerebral tumors so it is necessary a careful clinical observation in order to consider GH treatment in patients carrying *LZTR1* variants.

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P03.34B
Utility of MODY probability calculator among HNF1A- and GCK-MODY Polish patients (a retrospective analysis)

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Introduction: The most common form of monogenic diabetes is MODY. An easy-to-use MODY prediction model for identifying genetic-test indicated patient cases was developed in 2012: www.diabetesgenes.org/content/mody-probability-calculator. The aim of this study was to validate the utility of this tool with MODY patients in the Polish population.

Materials and Methods: Our MODY patient database was established 18 years ago at the Department of Metabolic Diseases, JUMC, Krakow, Poland and was based on typical clinical inclusion criteria. Patients in the database who provided all answers to the MODY calculator questionnaire --106 GCK-MODY and 92 HNF1A-MODY-- were included. The control group was established with 85 T1DM individuals and it does not include any T2DM patients due to insufficient number of sub-35-year-old T2DM cases in our outpatient clinic --as required in the calculator model.

Results: The mean predictive value using the calculator was 63.1% for GCK-MODY and 44.92% for HNF1A-MODY patients. This is in contrast with the mean calculator predictive value of 4.82% for T1DM patients. Only two T1DM patients out of 85 obtained a calculator predictive value higher than 25% -- the minimum suggested criteria for genetic testing referral. Only 11 GCK-MODY patients and 33 HNF1A-MODY patients received a sub-25% score. Sensitivity and specificity for both types of MODY were 77.78% and 97.65% respectively. GCK-MODY when compared to HNF1A-MODY obtained better sensitivity.

Conclusion: The model based on the Hattersley's group calculator reliably indicated genetic testing for GCK-MODY patients among our sub-population of Polish patients. The obtained results for HNF1A-MODY patients were also satisfactory.

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P03.35C
Assessment of genes known to be associated with MODY by next-generation sequencing

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MODY (Maturity Onset Diabetes of the Young) is a autosomal dominant heterogeneous form of diabetes mellitus which mainly affects children and young adults. The defect in beta cell function is the cause of MODY. There are several known subtypes of MODY and each subtype occurs by pathogenic variants of different genes. Approximately 80% of MODY occurs by GCK and HNF1A pathogenic variants in European population, but different ethnics may have different rates of subtypes. This study aimed to explain the genetic architecture of MODY-suspected 132 Turkish patients. In this study, genes known to be associated with MODY (*SLC161,KLF11,G6PC2,*

Genes	Mutations	Genotype	Classification	n	Frequencies (%)
GCK	p.Arg191Trp (c.571C>T)	Heterozygous	Pathogenic	2	1,51
	p.Gln38Pro (c.113A>C)	Heterozygous	Pathogenic	1	0,75
	p.Arg191Gln (c.572G>A)	Heterozygous	Pathogenic	2	1,51
	p.Thr228Met (c.683C>T)	Heterozygous	Pathogenic	2	1,51
	p.Asp364Glyfs) (c.1090_1091insGCTGCGACCCTCGACCACCG) Novel	Heterozygous	VUS	1	0,75
	p.Ala380Thr (c.1138G>A)	Heterozygous	VUS	2	1,51
	p.Arg37Trp (c.109C>T)	Heterozygous	Pathogenic	2	1,51
	p.Val227Met (c.679G>A)	Heterozygous	Pathogenic	1	0,75
BLK	p.Ala71Thr (c.211G>A)	Heterozygous	VUS	6	4,54
	p.Asp166fs (c.497delA)	Heterozygous	VUS	1	0,75
PDX1	p.Pro33Ala (c.97C>G)	Heterozygous	Pathogenic	2	1,51
RFX6	p.Glu75Gln (c.223G>C)	Heterozygous	VUS	1	0,75
	c.2393+8T>C	Heterozygous	VUS	1	0,75
G6PC2	p.Tyr207Ser (c.620A>C)	Heterozygous	DAP	2	1,51
HADH	p.Phe92Cys (c.275T>G)	Heterozygous	VUS	5	3,78
HNF1A	p.Ala15Thr (c.43G>A)	Heterozygous	VUS	1	0,75
HNF1B	p.His336Asp (c.1006C>G)	Heterozygous	Pathogenic	1	0,75
PDX1 *HADH*	p.Pro33Thr (c.97C>A) p.Gly34Ser (c.100G>A)	Compound Heterozygous	Pathogenic VUS	1	0,75
RFX6 *HNF4A*	p.Glu75Gln (c.223G>C) p.Gln10Terp (c.28C>T)	Compound Heterozygous	VUS VUS	1	0,75
G6PC2 *HNF4A*	p.Tyr207Ser (c.620A>C) p.Gln10Terp (c.28C>T)	Compound Heterozygous	DAP VUS	1	0,75
G6PC2 *INSR*	p.Tyr207Ser (c.620A>C) p.Val1012Met (c.3034G>A)	Compound Heterozygous	DAP VUS	1	0,75
SLC16A1 *GCK*	p.Tyr359Ser (c.1076A>C) p.Thr229Met (c.686C>T)	Compound Heterozygous	VUS Pathogenic	1	0,75
PAX4 HNF4A	c.539-5C>T p.Ile463Val (c.1387A>G)	Compound Heterozygous	VUS VUS	1	0,75
WILD TYPE				93	70,45
TOTAL				132	100

HADH,MOG,ZFP57,RFX6,GCK,PAX4,BLK,GLIS3,NEUROG3,GLUD1,INS,KCNJ11,ABCC8, HNF1A,PDX1,HNF1B, INSR,NKX2-2,HNF4A,FOXP3) were sequenced by using IonTorrent S5 and runs were then analyzed with bioinformatics pipeline. Of 132 patients, pathogencic, VUS(variant-unknown-significance), and DAP(disease-associated polymorphism) were detected in 39 patients. Additionally, a new variant identified in GCK is classified as pathogenic according to ACGM criteria and this variant was determined as de novo with the segragation analysis. The most common pathogenic variants were seen in *GCK* and the results are shown on the table. The novel variant detected in GCK results in premature stop codon because of frame shift mutation. The pathogenic variants detected in different genes revealed that NGS method is suitable to test genetic ethiology of multigenic diseases like MODY.

Table: Mutations and frequencies (%) in 39 patients.

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P03.36D
Molecular characterization of a cohort of MODY patients from the North of Portugal

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Introduction: Maturity-onset diabetes of the young (MODY) is a group of monogenic disorders of autosomal dominant transmission resulting from a primary defect in insulin secretion, associated with pancreatic β-cell dysfunction. It is the cause in approximately 2% of diabetic patients, but it is frequently misdiagnosed as type 1 or type 2 diabetes. Although fourteen different genes have been implicated, mutations in the glucokinase gene, *GCK*, and genes coding for hepatocyte nuclear factor 1α, 1β and 4α (*HNF1A*, *HNF1B* and *HNF4A*, respectively), are the most common causes of MODY, accounting for up to 70% of mutated alleles.

Methodology: 112 patients with criteria for MODY type diabetes, from adults Endocrinology or Genetics consultation, underwent molecular genetics testing at UBG. Molecular genetics studies were performed by Sanger sequencing of one or more of the following genes, according to clinical suspicion: *HNF1A*, *GCK*, *HNF4A* and *HNF1B*.

Results: We present the molecular characterization of 24 patients where a definite or probable causing mutation was identified, thus 21%. Fourteen (58%) are HNF1A-MODY, eight (33%) are GCK-MODY and two (8%) are HNF1B-MODY. Eighteen different mutations were identified - 10 in *HNF1A*, 6 on *GCK* and 2 on *HNF1B* - including 3 novel mutations: c.1146_1156del and c.1422_1424delGCCinsCAG in *HNF1A* and c.863T>C on *GCK*.

Conclusions: The prevalence of HNF1A-MODY and GCK-MODY in our cohort is much lower than the values described. The MODY spectrum in Portugal is expected to be different from the one reported in other populations and will be revealed after the study of remainder MODY genes.

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P03.37A
Mutations in the Hepatocyte Nuclear Factor 1 Alpha (*HNF1A*), 4 Alpha (*HNF4A*) and 1 Beta (*HNF1B*) in Maturity-Onset Diabetes of the Young in Croatia

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Introduction: Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes that is characterized by an early onset (usually before 25 years), autosomal dominant mode of inheritance and a primary defect in pancreatic β-cell function. MODY is a common form of monogenic diabetes and it may account for 1% to 2% of all diabetes cases in Europe. Many people with MODY are misdiagnosed with type 1 or type 2 diabetes.

Materials and Methods: All adult subjects with diabetes onset under age of 45 years and currently older than 18 years. Other inclusion criteria are: evidence of endogenous insulin secretion (fasting or random C-peptide ≥ 0.2 nmol/L) and negative glutamic acid decarboxylase antibodies (GADA), islet cell autoantibodies (ICA) and islet tyrosine phosphatase 2 (IA2). We analysed 44 patients for HNF1A-MODY, 14 patients for HNF4A-MODY and 8 patients for HNF1B-MODY. For identification of mutations in the coding and promoter region of analyzed genes, we used the Sanger sequencing method.

Results: We identified six different mutations in nine patients for subtype HNF1A-MODY. These mutations are located in exons 2, 3, 4 and 6, but the greatest number are found in exon 4, p.Gly292Argfs*25. For subtype HNF1B-MODY we identified one mutation, p.Val458Gly in exon 7. No HNF4A-MODY gene mutations were identified in any of patients analyzed.

Conclusions: Correctly identifying MODY has important implications for treatment, surveillance of complications and associated extra-pancreatic disorders, and identification of affected and at-risk family members.

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P03.38B
MODY genes *HNF1A, GCK* and *HNF4A* pathogenic variant spectrum in pediatric patients

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Introduction: Maturity onset diabetes of the young (MODY) is a rare form of diabetes which is caused by pathogenic variant in one of the thirteen known responsible genes, but mostly in 3 genes: *HNF1A, GCK, HNF4A*. Correct diagnosis might allow change treatment, especially when it is mistaken for type 1 diabetes. Here we analyzed genetic variants in Lithuanian pediatric patients with suspected monogenic diabetes.

Materials and Methods: We performed *GCK, HNF1A, HNF4A* genes Sanger sequencing for pediatric patients (0-18 years old) for whom there was mild hyperglycemia or were diagnosed type 1 diabetes with negative antibody markers (glutamic acid decarboxylase, insulin and insulinoma-associated-2 antibodies) in the year 2017-2018.

Results: 32 patients were analyzed for all 3 genes, or one of them depending on clinical situation. 50% of them had at least second degree relative with anamnesis of diabetes or hyperglycemia. For 8 of the patients we found *GCK* non-synonymous heterozygous variants which were previously reported as pathogenic. 2 patients had heterozygous variants in *HNF1A:* one with frameshift variant c.872dupC and another nonsynonymous variant c.809A>C which was not reported earlier. This variant changes amino acid in DNA binding region (p.Asn270Thr), cosegregates in patients family as patients father and grandmother (father's line) were diagnosed with diabetes. Also, as multiple *in silico* predictors showed as pathogenic variant, we ascertained variant as likely pathogenic.

Conclusion: In our study, only 1/3 of patients were proven for carrying a disease causing allele of all suspected MODY patients. One patient had novel variant with no previous report.

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P03.39C

Functional characterization of *HNF1A* variants identified in the Norwegian MODY diabetes registry can implement precision medicine in diabetes clinics

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Introduction: Variants in the hepatocyte nuclear factor-1alpha gene (*HNF1A*) can cause Maturity-Onset Diabetes of the Young (MODY3). MODY3 patients, who are often misdiagnosed as type1/type2 diabetes, are important to identify, since they can benefit from sulfonylurea treatment rather than insulin. The aim of this study was to functionally investigate possible pathogenic effects of 11 *HNF1A* variants from the Norwegian MODY registry, in order to provide a precise diagnosis and treatment.

Materials and Methods: The Norwegian MODY registry includes 2125 individuals with suspected MODY. *HNF1A* variants (Sanger identified) were investigated by transactivation (luciferase assay), protein expression (immunoblotting), DNA binding (electrophoretic mobility shift assay) and nuclear localization assays.

Results: All *HNF1A* variants investigated were classified as variants of uncertain significance. Variants p.(Ala116Thr), p.(Lys222del) and p.(Asn266Ser) showed low transcriptional activity (15-36%). p.(Ala116Thr) and p.(Lys222del) demonstrated low DNA binding (23% and 17%), and impaired nuclear localization. Moreover, p.(Lys222del) exhibited low protein expression (26%). Carriers of functionally impaired variants manifested with early diabetes-onset (<25 years). The autoantibody status was negative or not tested. Carriers of p.(Ala116Thr) and p.(Lys222del) had a family history of diabetes. The p.(Ala116Thr)-carrier had earlier been treated with sulfonylurea with no optimal effect. Her sister (also carrier), is treated successfully with sulfonylurea. The p.(Lys222del) and p.(Asn266Ser) patients are currently treated with insulin/metformin, and should be considered shifted to sulfonylurea. The remaining variants were either functionally normal or moderately impaired.

Conclusions: Functional investigation of *HNF1A* variants should support precision medicine in MODY3 patients.

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P03.40D

New generation sequencing as an effective method of diagnosing patients with various form of monogenic diabetes

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Introduction: Monogenic forms of diabetes represent 5-7% of all diabetes types. They are a heterogeneous group of disorders caused by mutations in single genes, most of which regulate the pancreatic β cells function. The aim of the study was to determine the genetic background in patients referred to the Outpatient Genetics Clinic of the Centre for Monogenic Diabetes in Lodz, Poland.

Materials and Methods: The study group consisted of 379 patients with suspected monogenic diabetes aged from 3 months to 38 years diagnosed from February 2017 to January 2019. They were referred based on the age of diagnosis, family history, autoantibodies status, preserved insulin secretion, different clinical course of diabetes or coexistence of other symptoms. The next generation sequencing (NGS) method was performed using a designed panel of 35 genes (SureSelect, Agilent) and the following platforms and programs: Variant Studio (Illumina®), PhenIX (Charité Universitätsmedizin Berlin) and IGV (Broad Institute).

Results: Various forms of monogenic diabetes were confirmed in 75/379 (19.8%) of patients. 40 pathogenic variants of the *GCK* gene (MODY2), 8 variants of the *HNF1A* gene (MODY3), 7 variants of the *HNF4A* gene (MODY1), 7 variants of the *BLK* gene (MODY11), 7 variants of the *KCNJ11* gene (PNDM - permanent neonatal diabetes mellitus), 5 variants of the *HNF1B* gene (RCAD - renal cysts and diabetes syndrome) and 1 variant of the *ABCC8* gene (PNDM) were identified.

Conclusions: The NGS method seems to be effective diagnostic tool for patients with monogenic diabetes. Supported by the grants No 2015/19/B/NZ5/02243, 502-03/2-159-02/502-24-307 and 502-03/2-159-02/502-24-306.

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P03.41A
Analysis of genes associated with the development of nephronophthisis

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Objectives: Nephronophthisis is an autosomal recessive disease genetically very heterogenous. Currently is described 25 genes responsible for up to 70% of the disease from which 30-40% is caused by *NPHP1* gene deletion. The main symptoms are kidney degeneration and fibrotic tissue formation, leading in renal failure often in childhood. Other less common symptoms include growth retardation, anemia and metabolic changes. The disease is caused by the presence of two causal mutations from parents, formation of disease *de novo* is rare. We describe three families their children have a defined clinical diagnosis of nephronophtisis.

Methods: We tested DNA isolated from peripheral blood and the molecular analysis of genes associated with nephronophthisis was performed by MLPA and custom designed NGS. Found causal mutations were verified by Sanger sequencing.

Results: By the analysis of 3 patients with clinical diagnosis of nephronophtisis we found homozygous causal mutations in two of them and two different heterozygous causal mutations (compound heterozygote) in one of them.

Conclusion: Molecular genetic testing confirmed a clinical diagnosis of nephronophtisis in all three patients. Follow-up segregation of causal variants in the patients' families identified healthy carriers of these variants and in one family we confirmed the diagnosis in affected siblings. The knowledge of causal mutations is important not only for confirmation of the diagnosis, but also for healthy carriers in case of pregnancy planning and possible prenatal testing.

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P03.43C
PEMT, PCYT1 A and B polymorphisms in children with obesity

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Introduction: Variations in *PCTY1A* and *B* genes associate with alterations of PCTY1 expression and of phosphatidylcholine (PC) synthesis. PC, the major glycerophospholipid in eukaryotic cells, can be synthesized by three pathways: the de-novo pathway, also known as Kennedy

pathway, the Lands cycle and the phosphatidylethanolamine methyl transferase (PEMT) pathway. PC biosynthesis is required for normal very low-density lipoprotein secretion from hepatocytes and generation of arachidonic acid (ARA); its inhibition may lead to excess storage of lipids within liver, contributing to the development of obesity-associated hepatosteatosis.

Aim: Whether PCTY1A, PCTY1A B and PEMT gene polymorphisms in obese children associate with obesity-related somatometric and biochemical parameters.

Material and Method: 200 obese children, aged 7 to 18 years old; BMI>+2SD; abdominal circumference above the 90th percentile; +/- HTA and dyslipidemia or insulin resistance. PCTY1A (rs 1580820) and PCTY1B (rs 4898190), PEMT(rs1109859, rs12103822, rs16961845, rs4244593, rs4479310, rs7214988, rs7946, rs8068641, rs936108, rs13342397, rs6502603) were analysed using next generation sequencing.

Results: PCTY1A, PCTY1B and PEMT rs1109859 variations were positively correlated with glycemia, cholesterol, HDLc, total LA, ARA, BMI, cardiac frequency, and arterial and systolic blood pressure. PCTY1A and PCTY1B polymorphisms were correlated with plasma choline, and possibly with plasma betaine and 5-methyl tetrahydrofolate.

Conclusion: Genetic variations involved in choline metabolism were associated with alterations in choline, folate, and cholesterol metabolism in obese children, and could play important roles in the severity of associated metabolic alterations. **Acknowledgement:** This work was performed at The Center of Genomic Medicine, POSCCE Project, SMIS:48749, and funded by POC Project Nutri-Gen, SMIS:104852.

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P03.44D
Detection of adenoma derived circulating cell-free DNA of human pituitary adenoma using semiconductor sequencing

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Introduction: Circulating cell-free DNA (ccfDNA) analysis has been lately used as a minimally invasive method in cancer diagnostics. Currently, there is a scarce amount of information on adenoma derived ccfDNA. We examined the possibility to detect pituitary adenoma (PA) derived ccfDNA, by performing targeted NGS on ccfDNA using semiconductor sequencing.

Materials and Methods: Blood samples used were obtained from five NFPA patients before PA surgery. Sixteen tumor specific mutations each in unique gene (discovered in exome sequencing) were targeted in ccfDNA.

Results: Sequencing coverage was 6191X on average (range 444 - 13026). In two mutation sites (two patients) the alternate allele was in less than in 10% of reads: *RYR1* (G/A, 2.27%), *MPRIP* (C/T, 3.65%). Five mutations (from two patients) had the alternate allele close to 50% of reads: *VPS13D* (C/T, 48%), *LDLRAD2* (C/T, 47%), *SPEN* (A/G, 49%), *GPATCH4* (C/T, 58%), *G6PC2* (C/G, 46%). The alternate allele was in less than in 0.1% of reads in nine mutations, however, the estimated background alternate allele rate in non-mutation sites also was below 0.1%.

Conclusions: Results indicate that while it is possible to detect somatic mutations found in adenoma in ccfDNA using semiconductor sequencing the source of mutated ccfDNA molecules should be further researched. Mutation rate in 50% of reads could be due to somatic mosaicism or due to increased tumor cell apoptosis/necrosis in these patients.

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P03.45A
Exome sequencing of non-functioning pituitary adenomas reveal tumors with high amount of somatic mutations and previously reported genes

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Introduction: The most common type of pituitary diseases is pituitary adenomas (PA). Although not undergoing

metastasis, PAs are responsible for increased mortality and morbidity. Genetic causes of PAs are known in a minority of cases: several genes are implicated in familial PA. Somatic mutations in *GNAS* and *USP8* are cause in subgroups of sporadic GH and ACTH secreting PA, respectively. Exome and genome sequencing is used to study PA genetics nowadays, but is hampered by heterogeneity of PAs.

Materials and Methods: Exomes (TruSeq Rapid Exome TargetedRegions v1.2) of normal - tumor DNA pairs of seven non-functioning PA patients were sequenced using Illumina NextSeq. Data were analysed using Isaac-Starling pipeline. Somatic mutations were reviewed with IGV2.2.4. Sanger sequencing validation was performed for a subset of mutations covering all PA patients.

Results: Various somatic mutation rate was observed in the exomes of sequenced tumors. Two PAs were hypermutated with 272 and 671 somatic mutations. Other five PA had a low somatic mutation rate between one to 12 somatic mutation per exome. *VPS13D* in hypermutated tumor and *RYR1* had somatic mutations and had been reported containing somatic mutation previously in GH secreting adenomas. Somatic mutations in *G6PC* and *SMARCAD1* previously have been detected in adenomas of different location.

Conclusions: Some PAs contain a significantly higher amount of somatic mutations than expected from non-metastasizing tumor. Somatic mutations in genes reported previously in connection with PA provide research targets for upcoming studies. The study was supported by ERDF grant 1.1.1.1/16/A/066.

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P03.46B
Tracing origin of cultured pituitary adenoma cells using "omics" analysis

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Introduction: Pituitary adenomas (PA) are benign tumours of the anterior pituitary that cause increased mortality and morbidity. Cell cultures derived from PA tissue can form free floating aggregates called pituispheres (PS) or adherent mesenchymal stromal cells (MSC). We studied genetic relationship between patients' germline DNA, tumour tissue somatic DNA, DNA of PS and MSC obtained from culture to trace origin of each type of cultured cells.

Materials and Methods: PA patients were enrolled to national biobank - Genome Database of Latvian Population from Pauls Stradins Clinical University Hospital where transsphenoidal surgery of PA was performed for all patients. Exomes (Illumina TruSeq_Rapid_Exome_TargetedRegions_v1.2) of germline, tumour somatic, PS and MSC were sequenced using Illumina NextSeq with 75bp paired end reads. Sequencing data were analyzed with Illumina Basespace Enrichment App (v3.0.0) aligning to human HG19 reference genome using Isaac Genome Alignment Software, variants called with Starling algorithm and variants annotated with Illumina Annotation Engine. Filtered variants were reviewed using IGV 2.3.14.

Results: PAs contain low amount of tissue specific mutations (median 4, range 2 - 6). Somatic mutations of the primary tumour can be detected in the respective PS, but not in the respective MSC. Genetic alterations of MSCs corresponded to mutations in PA patients' germline DNA.

Conclusions: Using exome comparison we were able to trace origin of PS and MSC cell cultures, showing that genome of PS represents genome of PA while MSC most likely represent normal cells of pituitary or surrounding tissues.

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P03.47C
Improved mapping quality and coverage in highly homologous *PKD1* gene enable high diagnostic yield in ADPKD

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is an adult-onset multisystem disorder characterized by cysts in the kidneys and liver. It is estimated that *PKD1* explains 85% and *PKD2* 15% of ADPKD cases. Genetic testing has become an important factor in the management of ADPKD. However, analysis of *PKD1* is technically challenging due to its large size, high GC-content and duplication of the first 33 exons with a high

degree of homology to pseudogenes (*PKD1P1-P6*). We evaluated the diagnostic yield and performance of our In-house panels for cystic kidney disease.

Materials and Methods: Next-generation sequencing (NGS) was performed using the IDT xGEN Exome Research Panel with added custom probes and the Illumina NovaSeq 6000 platform. This assay provides improved mapping quality and coverage of *PKD1* compared to other NGS methods assessed in our laboratory.

Results: *PKD1* provided both high mean coverage (199x) and excellent mapping quality with 99.5% of the target nucleotides covered at least 20x. In the study cohort of 131 index patients, a genetic diagnosis was established in 52% of cases with disease causing variants detected in 7 different genes. In 63% of the diagnostic cases the disease causing variant was identified in *PKD1*. Interestingly, 84% (n=36) of the variants were located in the duplicated region of *PKD1*.

Conclusions: Our results demonstrate that our in-house NGS platform is well-suited for clinical diagnostics with comprehensive coverage in difficult-to-sequence regions of *PKD1*. The method provides a cost-effective diagnostic tool to simultaneously diagnose various types of mutations.

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P03.48D
Detection of DZIP1L mutations by whole exome sequencing in two consanguineous families with polycystic kidney disease

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Autosomal recessive polycystic kidney disease (ARPKD) is an early onset cystic renal disease with an incidence of about 1 per 20,000 live births. It is most frequently caused by mutations in the *PKHD1* gene, encoding fibrocystin, but mutations in other cilia-related disease genes may mimic the phenotype. In 2017, Lu et al. reported homozygous mutations in *DZIP1L* located at 3q22.1, encoding the basal body protein DAZ interacting protein 1-like protein (DZIP1L) in seven children with ARPKD from four unrelated and consanguineous families. DZIP1L is located at the basal body of the primary cilium, and impaired function of DZIP1L is associated with a defect in the ciliary trafficking of polycystin-1 and polycystin-2. So far, no other *DZIP1L* mutations have been reported, and ARPKD caused by *DZIP1L* mutations is a rare form of polycystic kidney disease.We performed whole exome sequencing in two consanguineous families with three children with polycystic kidney disease, and identified two different and not previously reported *DZIP1L* mutations in homozygous form: c.193T>C; p.(Cys65Arg), and c.216C>G; p.(Cys72Trp). All three children are phenotypically characterized by enlarged echogenic kidneys with poor corticomedullary differentiation. No liver cysts were detected. Liver stiffness were evaluated in two of the children by transient elastography, and with normal result. Functional analyses of the p.Cys72Trp mutation indicate that this variant causes a disruption of a localization signaling or interaction domain of DZIP1L.

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P03.49A

Genetic analysis in pulmonary arterial hypertension Sicilian patients: preliminary results

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Background: Pulmonary arterial hypertension (PAH) is a progressive and fatal disorder associated with high pulmonary artery pressure. Most heritable PAH (75%) is caused by a pathogenic variant in *BMPR2*, pathogenic variants in other genes are considerably less common (1-3%).

Genetic testing enables early diagnosis and offers an opportunity for family screening.

Materials and Methods: We designed a target sequencing custom panel that includes 15 genes of interest. Genetic counselling was offered to every patients.

To identify genetic mutations and help make a precise diagnosis and family screening, we performed genetic testing by NGS in six patients with PAH.

Results: In three patients we identified variants classified according to ACMG Guidelines.

In a 47 years old female affected by familial PAH, we identified a heterozygous nonsense variant (c.2617C>T) p. Arg873* in *BMPR2* gene, classified as pathogenic.

In a 31 years old female affected by PAH with Pulmonary Capillary Hemangiomatosis, we identified a homozygous frameshift pathogenic variant (c.2666_2667insAATC) p. Lys891Aspfs* in *EIF2AK4* gene, classified as pathogenic and never described before.

In a 55 years old male affected by Pulmonary Arterial Hypertension, we identified a heterozygous missense variant (c.1151T>A) p.Val384Asp in *SMAD9* gene, classified as likely pathogenic and never described before. All variants were confirmed by Sanger sequencing.

Genetic counselling and molecular analysis was offered to high-risk relatives.

Conclusions: Our study confirm that NGS Target resequencing represents a valuable tool in PAH genetic testing. Analyses of PAH casual genes have a great help to clinical diagnosis and deep implications in patients management and disease treatment.

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P03.50B

Preparing for genomics: a retrospective audit of a metropolitan renal genetics clinic

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Introduction: Hereditary kidney conditions are rare but account for a significant burden on hospitals in both adults and children. Monash Health is a KidGen/ Australian Genomics Health Alliance/Melbourne Genomics Health Alliance flagship recruitment site, aiming to assess the impact of multidisciplinary renal-genetic clinics on patient care while providing access to whole exome sequencing (WES).

Materials and Method: This three year audit (2015-2017) of renal patients referred to the Monash Health genetics service provides a picture of the renal genetics landscape at the outset of renal flagship WES testing. The electronic medical record was searched using renal keywords; each file was manually assessed for inclusion and exclusion criteria. Data collection included referrer specialty, condition, family history, inheritance pattern, type and cost of genetic testing and impact of genetic testing on management and reproductive options.

Results: Through keyword matching 676 patients were identified, 86 met inclusion criteria; 59 (69%) were probands with a primary renal condition and 35 (40.1%) were referred by a nephrologist. Cystic renal disease was the commonest reason for referral. Genetic testing was arranged for 50 patients of whom 21 (42%) had informative results. Diagnosis rate improved from 2015 to 2017; cost per informative patient diagnosis was $2499.

Conclusion: Audit provides a snapshot of the utility of clinical genetics input in a renal genetics context at the outset of improved WES access. Fourteen referred patients were unaffected providing them with reproductive reassurance. Diagnosis rate improved from 2015 to 2017 as more molecular diagnoses were obtained, thus improving patient management.

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P03.51C

Molecular genetic analysis of Steroid Resistant Nephrotic Syndrome: Detection of a novel mutation

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Background: Nephrotic syndrome is one of the most common kidney diseases in childhood. About 20% of children are steroid-resistant NS (SRNS) which progress to end-stage renal disease (ESRD). More than 53 genes are associated with SRNS which represent the genetic heterogeneity of SRNS. This study was aimed to screen disease causing mutations within NPHS1 and NPHS2 and evaluate new potential variants in other genes.

Method: In first phase of study, 25 patients with SRNS were analyzed for NPHS1 (exon 2, 26) and all exons of NPHS2 genes by Sanger sequencing. In the second phase, whole exome sequencing was performed on 10 patients with no mutations in NPHS1 and NPHS2.

Result: WES analysis revealed a novel mutation in FAT1 (c.10570C>A; Q3524K). We identified 4 pathogenic mutations, located in exon 4 and 5 of NPHS2 gene in 20% of patients (V180M, P118L, R168C and Leu156Phe). Also our study has contributed to the descriptions of previously known pathogenic mutations across WT1 (R205C) and SMARCAL1 (R764Q) and a novel polymorphism in CRB2.

Conclusion: Our study concludes that mutations of exon 4 and 5 NPHS2 gene are common in Iranian and some other ethnic groups. We suggest conducting WES after NPHS2 screening and further comprehensive studies to identify the most common genes in the development of SRNS, which might help in Clinical impact on management in patients with SRNS.

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P03.52D
TNF-alpha gene polymorphisms influence disease phenotype in inflammatory bowel disease

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Introduction: Tumor necrosis factor (TNF) alpha is a major proinflammatory cytokine involved in the immune response in inflammatory bowel disease (IBD). IBD is composed of two main subtypes, namely Crohn's disease (CD) and Ulcerative Colitis (UC). There are multiple phenotypes of IBD in terms of disease extension, disease severity and extraintestinal manifestations (EIM). EIM in IBD are frequent and may significantly impact the quality of patients life. Our aim was to investigate two TNF-alpha gene SNPs for association with clinical manifestations in IBD patients.

Materials and Methods: The study included 142 IBD patients (78 CD, 43M/35F and 64 UC, 40M/24F), all of Romanian origin. EIM were documented in 34 patients (20 CD, 14 UC). The two SNPs of TNF-alpha gene rs361525 (-238G/A) and rs1800629 (-308G/A) were genotyped by TaqMan Allelic Discrimination Assay (7300 Real-Time PCR System, Applied Biosystems by Thermo Fisher Scientific, USA). Association tests for each polymorphism were performed with DeFinetti online software (http://ihg2.helmholtzmuenchen.de/cgi-bin/hw/hwa1.pl) and p values ≤0.05 were considered significant. Results. We found a significant association between -308A variant and the presence of EIM in both ulcerative colitis (p=0.0002, OR 8.51) and Crohn's disease (p=0.002, OR 5.19). For UC, -238A allele and the GA genotype were more frequent in patients with EIM than in patients without EIM (p=0.01, OR 27.03 and p=0.0009, OR 30.13 respectively). No other associations with clinical phenotype were observed.

Conclusion: TNF-α polymorphisms influence clinical manifestations of IBD in Romanian patients. These results should be confirmed on larger patients cohorts. Grant support: IDEI 311/2007.

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P04
Skeletal, connective tissue, ectodermal and skin disorders

P04.01A
Comprehensive re-assessment of causality of *ABCC6* missense variants associated with pseudoxanthoma elasticum

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Introduction: Pseudoxanthoma elasticum (PXE) is an autosomal recessive ectopic mineralization disorder caused by *ABCC6* mutations. Inconsistencies in variant interpretation raised the question whether all variants reported in literature or variant databases are truly disease-causing. Especially for missense substitutions it is challenging to

predict the impact on protein function. We therefore re-evaluated pathogenicity of all *ABCC6* missense variants.

Materials and Methods: The score-based variant classification system Sherloc was used to analyze 234 variants from literature, ClinVar and in-house patient screenings. Clinical and functional evidence were scored according to the rules and guidelines of Nykamp *et al*. (2017).

Results: Comprehensive classification revealed 74% variants of uncertain significance (VUS), 12% likely pathogenic, 10% pathogenic, 3% benign and 1% likely benign variants. VUS classification was further refined into truly uncertain variants (69% of VUS) and those leaning towards likely benign (1% of VUS) or likely pathogenic (30% of VUS). Taking the latter into account, 44% of *ABCC6* variants is (likely) pathogenic when considering all available population, clinical, experimental and *in silico* data. This is significantly different from ClinVar, where 87% of *ABCC6* missense variants are allegedly pathogenic and only 11% VUS.

Conclusions: Our results underline that variant classification should be done systematically and with caution, as it has important consequences for patients and heterozygous carriers. The high number of VUS confirms the need for functional testing to prove or refute their causality before returning them to patients.

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P04.02B
De novo pathogenic ABL1 variants cluster in a myristoyl-binding pocket

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Introduction: *ABL1* is a proto-oncogene encoding a nonreceptor tyrosine kinase. It is best known in the somatic *BCR-ABL* fusion gene associated with chronic myeloid leukaemia. Recently, Wang et al. described two germline missense variants in *ABL1* causing an autosomal dominant developmental syndrome (OMIM 617602) with congenital heart disease, skeletal malformations, and characteristic facies.

Materials and Methods: Patients with *de novo* heterozygous missense variants in *ABL1* were identified via trio WES through the DDD Study with Sanger confirmation via UK regional genetics laboratories. Clinical assessments of patients were carried out via regional clinical genetics services. To investigate *ABL1* kinase activity in vitro, HEK293T cells were transfected with plasmid constructs encoding wild-type or mutant *ABL1* cDNA. Phosphorylation of *ABL1*-specific substrates was measured by immunoblotting and potential for reversal investigated by treatment with imatinib. Bio-informatic analysis of gene-wide conservation and germline/somatic variation was performed.

Results: We identified three unrelated patients with *de novo* pathogenic missense variants in *ABL1*, including two novel variants. All variants cluster in the myristoyl-binding pocket of *ABL1* – a region critical for auto-inhibitory regulation of the kinase domain, which is subject to high missense constraint and evolutionary conservation. All patients recapitulate the phenotype of the *ABL1* developmental syndrome. Functional studies of mutant *ABL1* kinase activity are ongoing and will be presented.

Conclusions: We describe three new cases with pathogenic *ABL1* missense variants causing skeletal malformations and congenital heart disease. Mutations cluster around the myristoyl binding-pocket of *ABL1*, suggesting a gain-of-function mechanism due to loss of auto-inhibition.

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P04.03C
Rare loss-of-function variants in the Epidermal Differentiation Complex predispose individuals to Atopic Dermatitis

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The epidermal differentiation complex (EDC) includes over fifty genes encoding proteins involved in keratinocyte development. Of these genes, filaggrin (FLG) located on chromosome 1 q21, is the most studied in the context of skin barrier dysfunction. We investigated the frequency and effect of rare loss-of-function (LOF) variants in patients of a clinical study, VP-VLY-686-2102 (randomized, double-blind, placebo-controlled, in patients with chronic pruritus associated with AD) with 117 whole genome sequencing (WGS) samples. We have shown that 45/117 AD patients

carry significantly more, rare loss-of-function (LOF) mutations in the SFTP family of genes as compared to 55/316 in a control population (p-value = 0.000004). This group of EDC LOF (stopgain, frameshift) rare variants (EDC-LR) consists of 20 variants observed in the 45 AD patients resulting in a calculated Odds Ratio of 2.96 and a Relative Risk of 2.38. Among the detected LOF variants, there are 25 cases of FLG LOF mutations as defined by R501X (rs61816761), 2282del4 (rs558269137), other LOFs in FLG as well as LOFs in FLG2, HRNR, LCE4A, LCE5A, TCHH, TCHHL1 and other members of the EDC. We examine the regional accumulation of rare LOF variants in FLG region. For the entire EDC, we obtained a p-value of 4.7e-20, much lower than for FLG alone p-value of 4.5e-6, indicative of an even greater effect when analyzed jointly (entire family vs. FLG alone) in the AD context. The identified LOF variants within the region can serve as biomarkers as well as help delineate the genetic profile in AD patients.

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P04.04D
A balanced translocation t(2;7)(p21;p15) in three generations: Genome sequencing offers an opportunity to understand molecular etiology of Saethre-Chotzen/ Robinow-Sarouf syndromes

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Recent developments in genomic analyses have facilitated the precise mapping of translocation breakpoints associated with Mendelian disorders to decipher if the rearrangement is likely to contribute to the observed phenotype. We evaluated the phenotypic consequences of an apparently balanced translocation in a family affected by craniosynostosis and limb malformations presenting with a dominant inheritance pattern and variable expressivity. We applied large-insert jumping libraries to localize the breakpoints and confirmed these results with Sanger sequencing to characterize breakpoints of a t(2;7)(p21;p15), followed by gene expression studies to explore the functional impact of the rearrangement. Sequencing revealed five breakpoints, disrupting two genes, *HDAC9* and *MACC1*. We discovered altered expression of *TWIST1* located downstream of *HDAC9*. Our results suggest that an inversion in the HDAC9-TWIST region in 7p21.1 affects regulation of *TWIST*, thereby producing a skeletal dysplasia with intrafamilial variable expressivity. These analyses suggest that the mechanism of this rearrangement might involve alteration of regulatory sequences located at a distance in cis that dysregulate *TWIST* and insinuate *HDAC9* as a new disease locus producing craniosynostosis.

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P04.05A
Novel variant in *PRKAR1A* associated with brachydactyly type E without hormone resistance

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Introduction: Protein kinase A (PKA) is a tetramer containing a regulatory (R) subunit dimer locking 2 catalytic (C) subunits. The most abundantly expressed R-subunit is PRKAR1A, composed of a dimerization domain, an inhibitory site, and 2 cAMP-binding domains (CNB:A; CNB:B), each containing a phosphate binding cassette (PBC). Inactivating mutations in *PRKAR1A* cause acrodysostosis with hormonal resistance (ACRDYS1).

Patients and Methods: We present a case of a 15 year-old girl with clinical suspicion of pseudopseudohypoparathyroidism based on brachydactyly type E, severe scoliosis, and short stature without hormone resistance. Her

mother presented a similar phenotype. After discarding alterations in *GNAS, PTHLH*, *IHH* and *GDF5* (Sanger sequencing, MS-MLPA/MLPA), a skeletal dysplasia NGS panel was performed.

Results: A heterozygous novel variant in *PRKAR1A* [NM_002734.4:c.592G>C; p.(Gly198Arg)], bioinformatically predicted as likely pathogenic, was identified. Familial studies confirmed cosegregation (mother harbored; healthy grandparents did not harbor). Gly198 precedes the PBC of CNB:A and its substitution to Arg is predicted to affect function because: (1) it might affect cAMP binding to CNB:A, as the long side-chain of arginine will crash with its neighboring residues (inactivating mutation); (2) the introduction of the longer side-chain into the tight PBC space could change its conformation leading to an altered binding between the C and the R (activating/inactivating mutation).

Conclusions: *PRKAR1A* variants can lead to phenotypes milder than ACRDYS1 depending on the affected protein domain. Incomplete or partial PKA inactivation could lead to an isolated non-severe brachydactyly without hormone resistance.

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P04.06B
KIAA0753 mutations in skeletal ciliopathies: unveiling disease mechanisms

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Skeletal ciliopathies are a heterogeneous group of disorders caused by mutations in more than 25 genes, such as *KIAA0753*. Mutations in *KIAA0753* have been associated with Joubert syndrome, orofaciodigital syndrome, and moderate to fetal lethal skeletal dysplasia with narrow thorax and abnormal metaphyses.

We have previously reported patients with biallelic frameshift and nonsense mutations in this gene. We have also shown that *KIAA0753* is expressed in normal fetal human growth plate and immunohistochemistry of the growth plate of an affected fetus revealed an abnormal proliferative zone and a broad hypertrophic zone. To confirm the role of Kiaa0753 in skeletal ciliopathies, we used the zebrafish model. Phenotyping of zebrafish larvae carrying a nonsense mutation in homozygosity showed that mutant larvae presented with curved body, a typical ciliopathy phenotype, as well as abnormal cartilage patterning. *kiaa0753*-null zebrafish do not survive beyond the first week of development, suggesting that loss-of-function (LoF) mutations in *kiaa0753* are embryonic lethal. Interestingly, these *kiaa0753*-null larvae show loss of cilia and abnormal cell rearrangements, helping us understand how LoF mutations in *kiaa0753* affect cell organisation and embryonic development. In aggregate, our results show that Kiaa0753 is important for the early embryo development in zebrafish, in accordance with the phenotypes seen in patients. Future plans involve using this disease model to develop treatments for patients with *KIAA0753* mutations.

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P04.07C
p63 establishes epithelial enhancers at craniofacial development genes involved in orofacial clefting

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Transcription factor p63 is a key mediator of epidermal commitment, development, and differentiation. In humans, point mutations in the p63 coding region lead to developmental defects, including syndromic orofacial clefting. Notably, common risk variants located intronically in p63 contribute also to the risk of nonsyndromic cleft lip with/without cleft palate (nsCL/P), the most frequent isolated form of orofacial clefting. To date, our knowledge is limited about the role of p63 in human craniofacial development, due in part to a lack of tractable models. Here we investigated the role of p63 in human craniofacial development, and in the pathogenesis of nsCL/P. Using an inducible trans-differentiation model and systematic epigenomic sequencing (including ATAC-seq together with

ChIP-seq of p63 and H3K27ac) we show that p63 establishes enhancers at craniofacial development genes to modulate their transcription. Specific substitution mutations in the DNA binding or SAM protein interaction domain of p63 respectively eliminate or reduce establishment of these enhancers. Furthermore, using large-scale GWAS data we show that enhancers established by p63 are highly enriched for SNPs associated with nsCL/P. These orthogonal approaches indicate a strong molecular link between p63 enhancer function and nsCL/P, illuminating molecular mechanisms underlying this developmental defect and revealing vital regulatory elements and new candidate causative genes.

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P04.08D

Cleidocranial dysplasia: natural history of bone manifestations

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Cleidocranial dysplasia (CCD) is a rare autosomal dominant skeletal dysplasia whose most characteristic signs are the delayed closure of cranial sutures with wide fontanelle at birth, hypoplastic or aplastic clavicles, a short height and multiple dental anomalies. The natural history of CCD is poorly known. Isolated cases of patients with multiple fractures or osteoporosis have been described, without evaluating the other risk factors for bone fragility. The purpose of this study is to evaluate bone mineralization and the occurrence of fractures inpatients with CCD, children and adults. In an observational study, we collected clinico-radiological and genetics data, phosphocalcic balance, markers of bone remodeling and bone densitometry data of 45 CCD patients aged 9 months to 60.8 years (median = 15.4 years). 7% of patients evaluated had at least one fracture without trauma (3/45). 32% (13/41) patients have secondary hyperparathyroidism, a recognized risk factor for osteopenia due to increased bone resorption induced by parathyroid hormone and 20% (8/41) patients have vitamin D deficiency (25-OH D <20ng / mL). The analysis of BMD shows that the Z-score at the lumbar level of 38.7% of patients (12/31) is less than -2DS. These results indicate the need for active prevention of osteoporosis and fractures in patients with CCD, ensuring at an early age of sufficient intakes calcium and vitamin D, but also regular physical activity to allow optimal / maximal bone mass acquisition in adulthood.

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P04.09A

A novel mutation in COL2A1 leading to spondyloepiphyseal dysplasia congenita

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Introduction: Spondyloepiphyseal dysplasia congenita (SEDC, OMIM# 183900) is a rare autosomal dominant inherited chondrodysplasia. SEDC is characterised by dwarfism and skeletal abnormalities caused by mutations of the COL2A1 gene, which prevent bone growth. The most common features of SEDC are skeletal deformities such as short-trunk dwarfism, odontoid hypoplasia, cervical spine subluxation, scoliosis, kyphosis, lumbar lordosis, coxa vara, genu valgum, clubfoot, pes planus and metaphyseal changes. Cervical cord compression is the most hazardous skeletal deformity in patients with SEDC which requires special attention and management. Here, we present a mother and a son with SEDC due to a novel heterozygous mutation in SEDC.

Material and Methods: The patient was the first child of non consanguineous parents, born at 33 gestation week a

birth length of 39 cm (3-10th centile). Postnatal he was hospitalized in intensive care unit due to respiratory insufficiency. Physical examination revealed facial dysmorphic features and short neck. The mother has disproportionate short stature, facial dysmorphic features and operation scar due to hip dislocation. Her X ray showed platyspondyly, kyphosis, scoliosis, coxa vara and flattened epiphyses.

Results: Next-generation sequencing was performed on Illumina MiSeq (v1.9) platform using the virtual panel for skeletal dysplasia consisting of 130 genes. The novel heterozygous variant in COL2A1 (c.2006G>C) was detected both son and mother. Segregation within the mothers family showed the mutation was denova.

Conclusions: SEDC is a rare skeletal dysplasia. Targeted exome analysis has great importance in the fast and accurate setting in the diagnosis and avoid serious neurological deficits and or mortality.

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P04.10B
Novel variant in *COL2A1* gene causing spondyloepimetaphyseal dysplasia Strudwick type (SEMD-S)

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Introduction: Collagenopathies type II are rare autosomal-dominant conditions characterized by skeletal dysplasia, short stature and sensorial defects. More than 400 heterozygous mutations in type II collagen gene *COL2A1* have been identified, but only 5 of them are found in patients with spondyloepimetaphyseal dysplasia Strudwick type (SEMD-S), a rare phenotype of intermediate severity. We present a female patient with SEMD-S phenotype and a novel mutation in the *COL2A1* gene.

Patients and Methods: The patient presented at birth with severe short trunk-short limb dwarfism, short chest, coxa vara, clubfoot, cleft palate, conductive hearing loss and mild external hydrocephalus. Metaphyseal irregularities permitted clinical differentiation from SED congenita. Now, at the age of 7 years, her stature is significantly reduced and a waddling gait, genu valgum and lumbar lordosis developed. Hands and feet are normal. The genomic DNA from blood leukocytes of the patient was isolated and Next Generation Sequencing (NGS) analysis was done focusing on about 200 genes associated with skeletal dysplasias.

Results: NGS revealed a novel heterozygous missense variant (c.2177G>A, p.Gly726Asp) in exon 33 of the *COL2A1* gene, categorised as likely pathogenic according to ACMG/AMP classification. As glycine substitutions are typical for SEMD-S patients, it is highly probable that the identified variant is causative of the SEMD-S phenotype.

Conclusion: To our knowledge, this missense variant in *COL2A1* has not been reported before. It extends the mutational spectrum allowing for better genotype/phenotype correlation in patients with SEMD-S. Molecular genetic analysis is helpful for the diagnosis of skeletal dysplasias with overlapping phenotypes.

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P04.12D
Defining the clinical features associated with variants in *COL4A2:* case report and review of the literature

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COL4A2 encodes the α2 chain of type IV collagen, which assembles into heterotrimers with COL4A1 chains to form a component of the basal membrane. Heterozygous variants in *COL4A2* have been described in fewer than 20 families in association with familial cerebrovascular disease manifesting as porencephaly and intracerebral hemorrhage. Seizures, cortical malformations, lens opacities, hematuria and elevated creatine kinase have been reported. We present a 4-1/2 year old male with ischemic stroke and deep vein thrombosis, intracranial hemorrhage, systemic arterial involvement (tapering of the abdominal aorta, occlusion of the left common iliac artery, small right iliac artery and collaterals of the upper extremities) and mildly dilated aortic root and ascending aorta. Whole exome sequencing reported a maternally inherited variant in *COL4A2* (c.1396G>A, p.G466S). This variant is not reported in ClinVar but is reported in gnomAD with an allele frequency of $5.0x10^{-5}$. In silico models predict this variant to be damaging. The patient's mother is asymptomatic; brain MRI, echocardiogram and ophthalmology examinations are being coordinated. While intracranial hemorrhage has also been seen in other patients with *COL4A2* variants, systemic arteriopathy and mild enlargement of the aortic root have not been previously described. We review the literature and further define the clinical features associated with variants in *COL4A2* to include arteriopathy and aortic root dilatation.

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P04.13A
Next generation sequencing in neonates presenting with collodion baby syndrome

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Introduction: Collodion baby (CB) is a rare condition characterized by the presence of parchment or-cellophane-like collodion membrane encompassing the whole body. CB is a genetically heterogeneous disorder that usually presents with lamellar ichthyosis (LI) or congenital ichthyosiform erythroderma (CIE). Other less common disorders as self-improving collodion ichthyosis, Netherton syndrome, Gaucher disease type 2, Chanarin-Dorfman syndrome, ectodermal dysplasia or Sjögren Larsson syndrome may also be responsible for this phenotype.

Materials and Methods: Clinical exome sequencing (CES) was performed in 4 patients with referral diagnosis of CB using Illumina TruSight One Kit.

Results: CES analysis revealed mutations in *ALOX12B, SPINK5, NIPAL4* and *TGM1* genes. In patient 1 (P1), we found a homozygous mutation in the *ALOX12B* gene, known to cause self-improving collodion ichthyosis. This was consistent with a rapid resolution of almost all skin lesions. P2 and P3 developed Netherton syndrome. In P2 compound heterozygous mutations in the *SPINK5* gene confirmed the diagnosis. In P3 no causative genetic variations were found. In P4, presenting with CB evolving to LI, we detected two heterozygous variants, one in *NIPAL4* gene (likely pathogenic) and other in *TGM1* (uncertain significance).

Conclusion: Clinical evolution of CB is hard to predict as clinical presentation and severity may vary from mild to life-threatening. CES provided a timely and precise diagnosis in most of our patients which is important for treatment planning, disease prognosis and genetic counselling of affected families.

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P04.14B
IL11RA and pansynostosis

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The genetic background of craniosynostosis is complex and implicates multiple signaling pathways. The role of interleukin 11 receptor, alpha (IL11RA), a cell-derived cytokine, has been evidenced in the pathogenesis of multiple synostosis with autosomal recessive inheritance pattern (Craniosynostosis and dental anomalies, OMIM#614188). A Crouzon-like appearance with different types of craniosynostosis has been observed. Several causal missense and truncating variants have been reported. We present three additional cases with homozygous novel pathogenic and likely pathogenic variants in *IL11RA* (NM_001142784.2: c.598C>A, p.Pro200Thr; c.696C>A, p.Tyr232*; c.866A>G, p.His289Arg). All three patients presented with high intracranial pressure, a Crouzon-like phenotype and early closure of all cranial sutures (pansynostosis). Our cases, together with previous reports of *IL11RA*-related pansynostosis, suggest a key regulator role for IL11RA in the morphogenesis and maintenance of patency of all cranial sutures.

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P04.15C
Diagnostic value of targeted next-generation sequencing in craniosynostosis

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Background: Craniosynostosis (CS), the premature fusion of one or more cranial sutures, occurs either as an isolated malformation or in a syndromic form, representing a genetically heterogeneous and clinically variable group of disorders. Routine diagnostic screening of common craniosynostosis-associated genes enables to establish genetic aetiology in 21% to 62% depending on the size of the study, ethnicity of the population, and range of the molecular analysis.

Materials and Methods: We performed targeted next-generation sequencing in a group of 60 patients manifesting CS with negative results of molecular screening. We have designed a custom hybridisation-based panel, encompassing 61 genes and 11 SNPs chosen based on current knowledge about variants involved in craniofacial development using online databases. We sequenced captured and indexed libraries on Ion Torrent S5 sequencing system.

Results: This approach revealed pathogenic variants or probably pathogenic variants among patients presenting with different forms of CS. We found pathogenic and probably pathogenic variants or variants of unknown significance within the *ALX4*, *BMP4*, *CYP26B1*, *EDN3*, *EFNB1*, *EFTUD2*, *FGFR1, FGFR2, FGFR3, IFT122*, *IFT140*, *IHH, MEGF8*, *RECQL4*, *RUNX2*, *SKI*, *TCF12* genes.

Conclusion: We present an NGS targeted gene panel approach as a valuable diagnostic tool in the genetic testing of patients presenting with craniosynostosis.

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P04.16D

A genome-wide association study implicates *BMP7* as a risk factor for nonsyndromic metopic craniosynostosis

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Craniosynostosis (CS) arises from premature closure of one or more cranial sutures. Previously, we observed that *BMP2* (rs1884302) and *BBS9* (rs10262453) were independently associated with sagittal nonsyndromic CS (sNCS). In this study, we conducted a genome-wide association study (GWAS) for metopic NCS (mNCS). In an international sample of 228 non-Hispanic white case-parent trios, three

variants were genome-wide significant: rs781712 (P=4.788x10^{-9}; odds ratio (OR)=2.407) intronic to *SPRY3*; rs6127972 (P=5.611x10^{-9}; OR=2.257) intronic to *BMP7;* and rs62590971 (P=4.046x10^{-8}; OR=0.380) located ~155 kb upstream from *TGIF2LX*. The association for rs6127972 was replicated in an independent sample (194 unrelated mNCS cases, 333 controls, P=0.0042, OR=1.45) and in a meta-analysis, P = 3.11x10^{-9} (OR = 1.45). Rs1884302 and rs10262453, identified as risk factors from our previous sNCS GWAS, were also genotyped in our mNCS samples. Rs10262453 was borderline significant in the mNCS discovery and replications cohorts (P=9.216x10^{-5}, OR=1.716), (P=0.0003, OR=1.62) respectively, and had a meta-analysis P=1.330x10^{-7}(OR=1.62). Notably, the C allele for this variant was over-transmitted in mNCS probands, whereas the A allele was over-transmitted in sNCS probands. Functional assessment of rs6127972 using western blot and ELISA showed no difference in *BMP7* expression or protein levels in synostotic metopic versus open sutures. Data from luciferase studies suggested that the locus may act as a repressor element with the risk allele exerting stronger repression. Zebrafish transgenic analysis produced inconclusive results. Our findings implicate the *BMP/TGFβ* signaling pathway in midline NCS and support the role of *BBS9* locus in the etiology of metopic craniosynostosis. [Grants NIH X01HG008936 and R01DE016886]

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P04.17A

BMP2 or not *BMP2*? A *SMAD6*-related question in craniosynostosis

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Incomplete penetrance is a complicating factor in diagnosis and makes genetic counselling very challenging. Usually the mechanisms explaining the non-penetrance are not understood, with few epistatically-interacting genetic factors described in humans. One recent example of such a "two-locus" model is the proposal by Timberlake *et al* (*eLife*, 2016) of an interaction of *SMAD6* variants in midline craniosynostosis (premature fusion of the metopic and/or sagittal cranial sutures of the skull) with a common polymorphism near *BMP2*, to explain frequent non-penetrance in the parent transmitting the *SMAD6* variant. We aimed (i) to validate the findings in an independent, larger cohort, and (ii) to delineate the phenotype associated with *SMAD6* variants. We performed NGS-based resequencing of multiplexed PCR products of *SMAD6* in 800 unsolved patients with any type of craniosynostosis, and genotyped the rs1884302 *BMP2* polymorphism in *SMAD6*-positive individuals. We identified 18 different rare damaging *SMAD6* variants (2.25% overall), with the highest prevalence in metopic synostosis (~6%) and a ~23-fold enrichment of loss-of-function variants compared to gnomAD data (P<0.00001). This confirms that *SMAD6* variants increase the risk of craniosynostosis (especially metopic). Combining these data with a further 6 independently identified variants, 17/24 were transmitted from an unaffected parent but the *BMP2* genotype did not correlate with presence/absence of craniosynostosis. Hence further

work is needed to identify the factors accounting for reduced penetrance of *SMAD6* variants - both in craniosynostosis, and in the panoply of other phenotypes (including congenital heart disease, thoracic aortic aneurysm and intellectual disability), with which *SMAD6* variants have been associated.

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P04.18B
Chromosomal rearrangements and CNVs identified in craniosynostosis cases

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Craniosynostosis, the premature fusion of cranial sutures, is a common birth defect (~1:2500 newborns) occurring either as an isolated or syndromic form. Single nucleotide variants (SNVs) in known craniosynostosis genes i.e. *FGFR2, FGFR3* and *TWIST1* account for only 20% of cases. Besides these SNVs, a significant subgroup (15%) of patients with syndromic craniosynostosis show chromosomal rearrangements. Due to the small number of patients with identical chromosomal aberrations reported in the literature, it is often difficult to determine the critical region/gene which contributes to the phenotype and perform a specific diagnostic analysis. Thus, aCGH was chosen as genome-wide CNV screening approach for identification of CNVs in a cohort of 33 craniosynostosis patients.

In total we detected likely pathogenic CNVs in 18% of our cohort. These CNVs encompass known craniosynostosis genes like *MSX2* and *TCF12* and novel craniosynostosis candidate genes, respectively. Three patients carried unbalanced translocations and in additional three cases we detected interstitial gains and losses. Clinically these patients presented with isolated as well as syndromic forms of craniosynostosis.

Our results emphasize the importance of chromosomal rearrangements and CNVs as a genetic cause of craniosynostosis, particularly in case of unclear syndrome classification due to clinical variability. Furthermore, genes which reside in or are disrupted by rare CNVs represent candidate genes for craniosynostosis.

In addition to sequencing approaches CNV screening methods like aCGH should be included in the genetic testing algorithm for craniosynostosis patients. This will enable diagnosis in a significant portion of yet unsolved syndromic as well as non-syndromic cases.

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P04.19C
Biallelic loss of function mutations in CSGALNACT1 cause a mild skeletal dysplasia with joint laxity

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Biallelic loss of function mutations in *CSGALNACT1* were recently proposed to cause a prenatal onset mild skeletal dysplasia with joint laxity in a single 3.5 years old female patient. The phenotype in this patient reflects the skeletal dysplasia of *Csgalnact1-/-* mice and resembles the clinical and radiographic picture of a milder spectrum of Desbuquois dysplasia (Vodopiutz et al., 2017). *CSGALNACT1* encodes chondroitin sulfate N-acetylgalactosaminyltransferase-1 (CSGalNAcT-1, ChGn-1) which has a critical role in the biosynthesis of chondroitin sulfate and dermatan sulfate. Here we report on a 12 years old boy born from consanguineous parents with a mild skeletal dysplasia, short stature (-3SD), and joint laxity. Psychomotor development was normal. Whole exome sequencing revealed a homozygous 1bp-deletion in *CSGALNACT1* (c.372del, p.(His125Thrfs*9)). Segregation analysis confirmed both parents as heterozygous carriers. The identified variant is neither reported in gnomAD and dbSNP nor in the literature. It leads to a frameshift and premature stop codon and, thus, with high probability to loss of function of the corresponding protein. This is the second report of a patient with biallelic loss of function mutations in *CSGALNACT1* which underlines its role in mild skeletal dysplasia, short stature, and joint laxity without obvious additional organ manifestations and normal psychomotor development.

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P04.20D
Prenatal diagnosis of Desbuquois dysplasia by whole exome sequencing before the occurrence of specific ultrasound signs

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Osteochondrodysplasia is a group of hereditary disorders of the connective tissue, bones or cartilage that impair skeletal development with a prevalence of about 2 per 10 000 births. The genetic and phenotypic heterogeneity of this group of disorders is tremendous involving more than 300 genes that are responsible for more than 450 distinct conditions. One of them, Desbuquois syndrome dysplasia (DBQD) is a rare skeletal osteochondrodysplasia characterized by severe micromelic dwarfism, joint laxity with multiple joint dislocations, specific radiographic findings features and facial dysmorphism. DBQD is a very severe and often sometime lethal form of micromelic dwarfism. We report here a case for which whole exome sequencing allowed to perform an early prenatal diagnosis before the occurrence of characteristic ultrasound signs. Indeed, at 21 WG, the ultrasound scan confirmed that the fetus was very likely affected with an osteochondrodysplasia because all biometric parameters were below -4 SD associated with bilateral vertical talus. However, it was impossible, at that time, to pinpoint the exact diagnosis. Using exome sequencing, we evidenced two variants in *CANT1*, the gene responsible for DBDQ. The parents were informed that the fetus was very likely affected with DBQD at a gestational age of 24 weeks. The present case, is to our knowledge, the first one in whom a molecular prenatal diagnosis of DBQD is performed in the absence of relevant family history and before the appearance of evocative sonographic features. It highlights the utility of prenatal exome sequencing in performing a precocious diagnosis for severe fetal conditions.

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P04.21A
Vascular ehlers-danlos syndrome

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Introduction: Vascular Ehlers-Danlos syndrome is an autosomal dominant connective tissue disorder caused by mutations in *COL3A1* and *COL1A1*. It is considered as the most severe form of Ehlers-Danlos syndrome (EDS) due to the high mortality rate associated with the fragility of arteries and internal organs. Common symptoms include thin, translucent skin; easy bruising; characteristic facial appearance; and fragile arteries, muscles and internal organs. Treatment and management options are limited and focused on preventing serious complications. Here we present a 5 years old female with vascular type EDS due to a novel heterozygous mutation *COL3A1*.

Materials and Methods: The patient was the first child of non-consanguineous healthy parents, born at term with a birth weight of 2600gr (3-10th centile). Easly bruising even with small traumas have been noticed from the infancy period. She also had difficulty in wound healing. Physical examination revealed wide forehead, deep-set eyes, thin translucent skin with multiple bruising and atrophic scars, joint laxity, and profound superficial veins.

Results: Next-generation sequencing was performed on Illumina MiSeq(v1.9) platform using the virtual panel for collagenopathy consisting of 51 genes. The heterozygous variant in *COL3A1*(c.3563G>A) was detected. Segregation within the family showed that the variant is de-novo.

Conclusions: Although vascular type EDS is a rare genetic disease, it should be kept in mind in patients with easy bruisable skin and atrophic scar. Accurate and early diagnosis have great importance in preventing serious life threating complications.

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P04.23C
Homozygous mutations of EGFR gene as a cause of lethal syndrome with progeroid features in two Roma families

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EGFR gene encodes an epidermal growth factor receptor which is important in cell proliferation and differentiation. So far, only rare reports of germline mutations in the EGFR gene as a cause of monogenic disease in children are available. Our patient was a young pregnant woman of Roma origin. Her previous pregnancy was complicated by polyhydramnios and IUGR and her daughter was born in the 31st week of pregnancy, birth weight 660g. Child had no organ malformation but very fragile skin and died after 46 days because of multiorgan failure. The current pregnancy was also complicated by severe polyhydramnios, there were hyperechogenic kidneys and bowels and IUGR. The boy was born in the 30th week with birth weight of 760g. The boy had progeroid features with lack of subcutaneous fat and very thin and fragile skin. He died in 34 days because of metabolic dysbalance. Whole exome sequencing revealed presence of homozygous mutation c.1283G>A (p.Gly428Asp) in the EGFR gene in both deceased siblings, both parents are heterozygous carriers. Thirteen years ago, we examined Roma family in which 3 children died in early infancy with very similar clinical features. The diagnosis of Netherton syndrome was considered, but wasn't confirmed. With our new experience we tested mutation of EGFR gene in one of these deceased children. Homozygous mutation c.1283G>A was confirmed by Sanger sequencing. Mutations of EGFR gene should be considered in a differential diagnosis of progeroid syndrome especially in Roma families. Our report describes features of these children. Dedication: RVO-VFN 64165/2012

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P04.24D

Tessier no. 4 facial cleft leading to the diagnosis of familial vascular Ehlers-Danlos syndrome

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The parents of a girl born with Tessier no. 4 facial clefting sought genetic counselling concerning recurrence risk in the next pregnancy. The pattern of the facial clefting and a constriction ring on the right leg raised the suspicion of amniotic band to be the cause. Trio exome analysis was performed to best possible rule out other causes. A maternally inherited substitution of glycin to serin in the triple helix domaine (COL3A1:c.2689G>A;p.Gly897Ser) was identified in the COL3A1-gene, raising the suspicion of vascular Ehlers-Danlos syndrome. The family history was without vascular catastrophes, but one affected had severe bleeding after a gynecological surgery. Some carriers of the variant had easy bruising, whereas others did not. It is well known that substitutions of glycin to serin in the triple helix domaine lead to a better prognosis and a milder phenotype than other glycin substitutions in vascular Ehlers-Danlos Syndrome, which most likely explains the mild vascular phenotype in this family. This case reminds us that some collagen diseases increase the risk of amniotic banding, the risk being estimated to approximately 1 %. The other way round, the risk of a collagen disease in case of amniotic banding is to the best of our knowledge unknown. We suggest that genetic analysis for selected collagen diseases should be done in case of amniotic banding.

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P04.26B

Natural exon skipping reveals that antisense oligonucleotide-mediated exon skipping should be directed at the recessive type of dystrophic epidermolysis bullosa

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Introduction: Dystrophic epidermolysis bullosa (DEB) is a devastating genetic blistering disease affecting skin and mucous membranes. DEB is caused by pathogenic variants in the *COL7A1* gene encoding type VII collagen, and can be inherited dominantly or recessively. The structure of *COL7A1* makes it an attractive candidate for exon-skipping therapy. Recently, we demonstrated promising proof-of-principle for antisense oligonucleotide (AON)-mediated exon-skipping as a systemic therapeutic approach for DEB. However, it is unclear what phenotypic effect may be expected from exon-skipping and which patient groups may benefit the most.

Patients and Methods: To answer these questions, we studied new clinical and molecular data on seven patients from the Dutch EB registry and reviewed the literature on pathogenic *COL7A1* variants inducing 'natural exon-skipping'.

Results: We found that the natural skipping of certain exons led to disease in a heterozygous state, while the skipping of other exons only led to disease if combined with a pathogenic variant on the other *COL7A1* allele. The dominant DEB phenotypes associated with heterozygous exon-skipping could not be distinguished from dominant phenotypes caused by heterozygous *COL7A1* variants not inducing exon-skipping. Phenotypes associated with recessive exon-skipping mutations were, however, on average relatively mild in the spectrum of recessive DEB.

Conclusions: For dominant DEB, AON-mediated exon-skipping is unlikely to make a clinical difference. In contrast, we anticipate that exon-skipping has the potential to induce a clinically relevant improvement of the devastating recessive DEB phenotype, especially the types caused by bi-allelic null variants.

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P04.27C
Targeted next-generation sequencing in the diagnosis of facial dysostoses

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Background: Facial dysostoses (FD) encompass rare and heterogeneous congenital craniofacial anomalies subdivided into mandibulofacial dysostoses (MFDs) and acrofacial dysostoses (AFDs). Both MFDs and AFDs result from impaired migration of neural crest cells to the pharyngeal arches and the face during embryogenesis. The main clinical features observed in affected patients are maxillary, malar and mandibular hypoplasia, cleft palate, and/or ear defects. In AFDs, limb defects are present as an additional feature.

Materials and Methods: To determine the molecular aetiology of FD, we performed a custom amplification-based panel of 37 genes (145.5 kb, 761 amplicons) by next-generation sequencing on Ion Torrent S5 sequencing system. We analysed a group of 25 patients affected by variable forms of FD and validated clinically relevant variants by conventional sequencing in both affected individuals and their parents.

Results: Testing with targeted next-generation gene panel revealed pathogenic or probably pathogenic variants in 14 out of 25 patients presenting with different forms of FDs. We found either novel or known variants within the *ALDH1A1*, *ALX1*, *DHODH*, *EFNB1*, *EFTUD2*, *FGFR1*, *SF3B4*, *SRCAP*, *TCOF1*, and *ZSWIM6* genes.

Conclusion: We proved the efficiency and clinical utility of the designed gene panel. The targeted strategy presented here is a suitable and helpful approach in the genetic diagnostics of variable forms of FD.

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P04.29A
Novel mutations in autosomal dominant inherited syndromes with cutaneous manifestations

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Introduction: Molecular diagnosis provides useful information for disease prognosis and genetic counseling, especially when cutaneous changes are result from an

underlying hereditary tumor syndrome. We present four solved cases of autosomal dominant hereditary disorders displaying skin manifestations.

Materials and Methods: The cases consist on a 28 yo woman with multiple trichoepitheliomas (Brooke-Spiegler syndrome); a 10 yo girl with connective tissue *nevi* and osteopoikilosis (Buschke-Ollendorff syndrome); and two patients with multiple cutaneous leiomyomas (Reed syndrome), a 31 yo woman with cutaneous/uterine leiomyomas and renal cancer and a 38 yo man with cutaneous leiomyomas whose mother and sister had uterine lesions. All had a family history of the condition compatible with an autosomal dominant inheritance pattern. Disease-associated genes were analysed using PCR and direct sequencing and deletions/duplications were detected by MLPA.

Results: Pathogenic mutations were identified in all cases, three of them being novel. Patients with Brooke-Spiegler and Buschke-Ollendorff syndrome had non-sense mutations in respectively *CYLD* (p.Gly282Ter) and *LEMD3* (p.Trp581Ter), while Reed syndrome patients carried complete or partial *FH* deletions. This novel partial deletion extends 1.9 kb and its breakpoints are located within exon 2 and intron 2, disrupting the *FH* gene. Genetic counseling was performed and relatives at risk were offered direct molecular testing. Positive cases were further examined by imaging techniques to assess the presence of other lesions or malignancies.

Conclusions: Molecular testing confirmed the hereditary syndromes initially diagnosed and allowed to adopt adequate prevention and treatment measures in those positive cases with an increased risk of internal cancer.

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P04.30B

Two cases Ehlers-Danlos syndrome, kyphoscoliotic type, 2 caused by *FKBP14* mutation

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Introduction: *FKBP14*-related Ehlers-Danlos syndrome (EDS) is an extremely rare recessive connective tissue disorder. The poor prognosis is anticipated due to typical features of the syndrome and possible potentially life-threatening vascular complication in childhood and atlantoaxial instability.

Materials and Methods: We have observed clinical and genealogical examination of two patients (14 month-old boy and 11 month-old girl) with unusual phenotypes: the evaluation confirmed following symptoms: generalized muscle hypotonia, progressive kyphoscoliosis, and joint hypermobility. Echocardiogram and hearing was normal. Boy's ultrasound scan showed expansion of the axillary vein and girl's one showed ectasia of the internal jugular vein. Patients underwent genetic testing via high-throughput sequencing followed by capillary Sanger sequencing.

Results: In 2 patients from 2 unrelated Russian families identified homozygosity for a 1-bp insertion within a 5C-nucleotide repeat in exon 3 (c.362dupC, NM_017946.2) of the *FKBP14* gene, causing a frameshift predicted to result in a premature termination codon (Glu122ArgfsTer7). Their unaffected parents were heterozygous of the mutation.

Conclusion: Clinicians should give more consideration to rare genetic syndromes, especially in the case of symptoms from different clinical areas. Genotype/phenotype association studies will be necessary to elucidate further the cause of the variability of the disease severity. As it has early clinical manifestations, *FKBP*14-related EDS is still a challenge and the key issue for its effective follow-up that includes cardiovascular monitoring that is cerebral, thoracic, abdominal MRA, and cervical dynamic radiograph. The 362dupC mutation was linked to the same haplotype in all individuals despite their geographically diverse origins, suggesting a possible founder event

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Bi-allelic mutations in *LSS*, encoding lanosterol synthase, cause autosomal-recessive hypotrichosis simplex

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Hypotrichosis simplex (HS) is a rare form of hereditary alopecia characterized by childhood-onset of diffuse and progressive scalp and body hair loss. Although research has identified a number of causal genes, genetic etiology in about 50% of HS cases remains unknown. The present report describes the identification via whole exome sequencing of five different mutations in the gene *LSS* in three unrelated families with unexplained, potentially autosomal recessive HS. Affected individuals showed sparse to absent, lanugo-like scalp hair, sparse and brittle eyebrows, sparse eyelashes and body hair. The *LSS* gene encodes lanosterol synthase (LSS), which is a key enzyme in the cholesterol biosynthetic pathway. This pathway plays an important role in hair follicle biology. After localizing LSS protein expression in the hair shaft and bulb of the hair follicle, the impact of the mutations on keratinocytes was analyzed using immunoblotting and immunofluorescence. Interestingly, wild-type LSS was localized in the endoplasmic reticulum (ER), whereas mutant LSS proteins were localized in part outside of the ER. A plausible hypothesis is that this mislocalization has potential deleterious implications for hair follicle cells. Immunoblotting revealed no differences in the overall level of wild-type and mutant protein. Analyses of blood cholesterol levels revealed no decrease in cholesterol or cholesterol intermediates, thus supporting the previously proposed hypothesis of an alternative cholesterol pathway. The identification of *LSS* as causal gene for autosomal recessive HS highlights the importance of the cholesterol pathway in hair follicle biology, and may facilitate novel therapeutic approaches for hair loss disorders in general.

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P04.32D
Phenotypic presentations of Hajdu-Cheney syndrome according to age - 5 distinct clinical presentations

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We present five Danish individuals with Hajdu-Cheney syndrome (HJCYS) (OMIM #102500), a rare multisystem skeletal disorder with distinctive facies, generalised osteoporosis and progressive focal bone destruction. In four cases positive genetic screening of exon 34 of *NOTCH2* supported the clinical diagnosis; in one of these cases, mosaicism was demonstrated, which, to our knowledge, has not previously been reported. In one case no genetic testing was performed since the phenotype was definite, and the diagnosis in the mother was genetically confirmed. The age of the patients differs widely from seven to 56 years, allowing a natural history description of the phenotype associated with this ultra-rare condition. The evolution of the condition is most apparent in the incremental bone loss leading to osteoporosis and the acro-osteolysis, both of which contribute significantly to disease burden.

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P04.33A
Design of a candidate smMIPS sequencing study in patients with hereditary angioedema of unknown cause (U-HAE)

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Hereditary angioedema (HAE) is a rare, potentially life-threatening disease with an estimated prevalence of 1:50,000 characterized by recurrent episodes of subcutaneous or submucosal swellings. Transmitted as an autosomal dominant trait, there are five types of HAE with known genetic defects. While HAE with decreased C1-Inhibitor can be explained by mutations in *SERPING1*, only about 20-25% of the cases of HAE with normal C1-Inhibitor can be explained by mutations in *FXII*. But two recently discovered mutations in *ANGPT1* and *PLG* have been linked to two further forms of HAE with normal C1-Inhibitor. Despite these successes in identifying causes of HAE, there still remains a significant fraction of patients with the diagnosis of so called HAE with unknown cause (U-HAE). Aiming to identify potential new disease-causing genes that may play a role in the development or clinical heterogeneity of HAE, we performed multiplex targeted sequencing using the single-molecule molecular inversion probes (smMIPs). Our smMIPs panel comprises 29 genes that were prioritized by systematic literature research and pathway analysis and are mainly related to known disease-causing genes and pathways in HAE. The panel was established on a MiSeq platform where we achieved a coverage ≥100x for 95% of all exons in 27 out of 29 selected genes. Sequencing in our patient cohort of 125 HAE patients with U-HAE is currently ongoing on a Illumina HiSeq platform and results will be presented at the upcoming conference.

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P04.34B

Expanding the phenotype of Pseudoxanthoma elasticum with hereditary spastic paraplegia

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Background: Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder characterized by ectopic mineralization and fragmentation of the elastic fibers of connective tissues. *ABCC6* has been the only identified gene, but up to 18% of cases remain genetically unsolved with one or no pathogenic variant in *ABCC6 (Legrand et al, 2017)*. The corresponding negative French PXE cases were genetically explored for new PXE gene(s).

Methods: Exome sequencing was performed in one *ABCC6* negative PXE family with additional features, to search for pathogenic variants. We identified a candidate gene (CG) in a congruent metabolic pathway, and sequenced 46 additional *ABCC6* negative PXE index cases (IC). An in vitro biochemical assay was carried out to determine the pathogenicity of identified missense variants and a clinical description of positive cases was performed to refine their phenotypic spectrum.

Results: Out of 47 PXE IC, 3 harbored 2 pathogenic variants in our CG. Histologically-confirmed skin lesions, and diminished visual acuity due to maculopathy evoked the diagnosis of PXE. The association with neurological symptoms was strikingly present in the 3 cases, evoking a phenotype of PXE "plus" neurological symptoms. Biochemical analyses confirmed loss of activity of the corresponding mutated enzyme.

Conclusion: Our CG is mutated in 6.4% of our unsolved PXE cases and should be systematically sequenced in suspected PXE cases with neurological findings: spastic paraplegia, dystonia, cognitive impairment, peripheral neuropathy and brain MRI abnormalities. The mechanism leading to overlapping phenotypes for these 2 genes remains to be explored to explain their role and link in mineralization.

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P04.35C

Post-zygotic *MTOR* mutations and Ito hypomelanosis: phenotypic spectrum and ultrastructural characterization

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Germline MTOR mutations are associated with Smith-Kingsmore syndrome (SKS), whereas somatic mutations are reported in patients with hemimegalencephaly/focal cortical dysplasia; only few presented with linear depigmentation. Here, we report clinical manifestations in 12 sporadic cases with Ito hypomelanosis and post-zygotic mutation of *MTOR*.

We performed exome analysis or ultra-deep targeted sequencing of *MTOR* gene on hypopigmented skin biopsy, and performed precise clinical reappraisal. In two patients, we analyzed the phenotype histologically and ultrastructurally.

We identified 9 *MTOR* post-zygotic mutations including six novel. Two were recurrent. Clinically, patients had blaschkolinear hypopigmentation (11/12), epilepsy (9/12), macrocephaly (8/12), hemi-hypertrophy (7/12), and severe intellectual disability (7/12), 2 have iria heterochromia, and one nephromegaly. Primary fibroblasts (p.Glu2419Lys) showed activation of PIK3K-AKT pathway. Skin biopsies showed a decreased number of melanocytes melanosome immaturity in melanocytes and a decreased number of melanosomes in keratinocytes in hypopigmented areas.

Although somatic *MTOR* mutations have already been reported in Ito's hypomelanosis, this is the first clinical-genetic characterization of this association. Neurologically, there are analogies with SKS and hemimegalencephaly. Reduction of intrakeratinocyte melanosomes had already been reported in Ito's hypomelanosis, but without genetic study results as well as in hypochromic spots of tuberous sclerosis (TS), which is due to mutations of mTOR repressor TSC1/2. We found that tuberous sclerosis and MTOR-related hypomelanosis shared common pathophysiology process, with a lack of maturation of melanosomes. Importantly, no renal involvement known in TS has been identified in cases with *MTOR* mutations. Therapeutic use of rapamycin, a MTOR inhibitor, is therefore possible.

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P04.36D

Molecular analysis of ALPL gene in Russian cohort of patients with suspected hypophosphatasia

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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). The overall prevalence of severe HPP is range from 1/100 000 to 1/300 000. Mild forms of HPP are more frequent than severe forms - expected prevalence can reach 1/6000 in Western populations. Russian prevalence of mild and severe HPP is still unknown. Genetic analysis provides determining of diagnosis in cases with suspected HPP.

Materials and Methods: We analyzed genomic DNA samples from 112 unrelated individuals with signs of HPP (low and/or recurrent low levels of ALP, low growth, recurrent fractures and others). 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: Studied group included 66 males and 46 females (mean age 9yo, range from 1 month to 66yo). Low ALP (age- and sex-dependent reference range) was founded in 79% (89/112). We detected 15 pathogenic mutations (13% detection rate): 13 in heterozygous and 2 in compound-heterozygous. 3 novel missense variants were founded. Most frequent variant was p.E191K in exon 6. The prevalence of this mutation was 6,25% (7/112) in our study whereas gnomAD prevalence is 0,25%.

Conclusions: We presented the data of prevalence mild HPP in Russian cohort of patients with suspected HPP. Mild HPP was founded in 87% positive cases (13/15). Variant p.E191K in *ALPL* is common for Russian population.

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P04.37A

Recurrent somatic *IDH1* mutation in an adult with Maffucci syndrome

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Background: Maffucci Syndrome is a rare, highly variable, somatic mosaic condition, caused by recurrent mutations in either *IDH1* or *IDH2*. Features include benign enchondroma and spindle cell haemangioma, with a recognized increased risk of various malignancies. Fewer than 200 cases have been reported, therefore accurate estimates of malignancy risk are difficult to quantify and recommended surveillance guidelines are not available. *IDH1* and *IDH2* mutations are also implicated in a variety of other benign and malignant tumours. Recently, the FDA approved specific *IDH1* and *IDH2* inhibitors for use in individuals with relapsed acute myeloid leukaemia.

Methods: An adult male was assessed via the Austin Health Clinical Genetics Unit, after he presented with soft palpable lesions on the left upper limb. Imaging and histopathology raised the possibility of Maffucci syndrome. DNA was extracted from peripheral blood lymphocytes and from surgically resected tissue. Sanger sequencing and Droplet-digital PCR analysis of the *IDH1* gene was performed.

Results: Imaging and histopathology results were virtually pathognomonic of Maffucci syndrome. We identified a recurrent, somatic mosaic c.394C>T (p.R132C) mutation in exon 5 of *IDH1*, in DNA derived from haemangioma tissue (~ 17% mutant allele frequency) that was absent in DNA derived from blood. This mutation is a well-established cause of Maffucci syndrome.

Conclusion: We present an instructive case of a rare condition, and explore the potential therapeutic benefits and risks of *IDH1* and *IDH2* inhibitors in this disorder.

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P04.38B

FBN1 gene mutations in 26 Hungarian patients with suspected Marfan syndrome or related fibrillinopathies

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Introduction: Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder with cardiovascular, ocular and musculo-skeletal system involvement. *FBN1* gene mutations lead to MFS and related fibrillinopathies. In this work we describe clinical and molecular data of 26 unrelated individuals with suspected MFS who were referred to our laboratory for *FBN1* gene mutation analysis.

Materials and Methods: Genomic DNA samples and in one case RNA sample isolated from dermal fibroblasts were analyzed by next generation sequencing (NGS) and Sanger sequencing methods.

Results: We identified 23 causative or potentially causative (including variants of uncertain significance) *FBN1* variants, seven of them was novel (~30%). About 30% of the cases were sporadic. Missense mutations were detected in 69.6% (16/23), the majority of them were located in one of the cbEGF motifs and ~70% of them substituted conserved cystein residues. Small deletions/duplications accounted for 13% of the cases (3/23), while splice site variants were identified in 17.4% (4/23). In three unrelated patients a low frequency (<1%) recurrent silent variant (c.3294C>T (p.Asp1098=) was identified. *FBN1* mRNA analysis showed that the mutation does not lead to aberrant splicing, based on available data the variant was classified as benign.

Conclusions: *FBN1* mutations were associated with MFS in the majority of the patients, in two cases with severe and early onset manifestation of the syndrome.

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P04.39C
Identification of a novel two-exon deletion of *FBN1*gene in a patient with Marfan syndrome and homocystinuria

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Introduction: Marfan syndrome (MFS) is an autosomal dominant multi-system disorder of connective tissue with high clinical heterogeneity. Some characteristic features of the syndrome, such as ectopia lentis, long-bone overgrowth, a high arched palate, a crowded dentition, scoliosis, kyphosis and arachnodactyly overlap with those seen in homocystinuria. Co-occurrence of these disorders can be observed in a number of patients. MFS is mainly caused by mutations in the fibrillin-1 gene (*FBN1)*, mapped to chromosome 15q21.1. Majority of classic MFS patients bear small alterations, however, a smaller number of patients have larger genomic rearrangements in *FBN1* gene.

Materials and Methods: After negative results of systematic sequencing of *FBN1*, *TGFBR1* and *TGFBR2* genes, an MLPA (P065-P066 MRC-Holland) analysis was performed in a 21 years old female patient suffering from Marfan syndrome based on Ghent criteria and homocystinuria.

Results: In contrast to the generally detected large deletions, a novel two-exon deletion (exon 46-47) has been observed in our patient. This results in the loss of 31-32nd calcium binding EGF-like domain which is responsible for the development of classic Marfan phenotype.

Conclusions: Less than 10% of the disease causing mutations are copy number alterations, in which single or multiple exon deletions can be detected by MLPA in a cost-effective manner even in the NGS era. Our case points out the importance of testing the MFS patients for homocystinuria, since an elevated level of homocysteine is associated with the risk of severe cardiovascular manifestations, though at present only a few guidelines recommend testing for homocystinuria.

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P04.40D
A fibrillinopathy the marfanoid-progeroid-lipodystrophy syndrome - in mother and daughter

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Introduction: Marfanoid-progeroid-lipodystrophy syndrome (MPLS) is a very rare genetic disorder with clinical features overlapping those of congenital Marfan syndrome - ocular, cardiovascular and skeletal manifestations, progeroid syndromes - progeroid appearance of the face and body not associated with other manifestation of early aging and lipodystrophy with the extreme congenital lack of subcutaneous fat tissues not associated with metabolic disturbances. To our knowledge only seven patients with this syndrome were reported so far. In all patients mutation in exon 64 of the *FBN1* gene was detected.

Patients and Methods: We present clinical evaluation of mother and daughter in whom the same mutation Trp2756Ter in exon 64 of the *FBN1* gene was detected using next generation sequencing method (NimbleGen SeqCap Target Enrichment Roche) with panel of genes associated with craniosynostosis genes (developed in the Department of Medical Genetics of the Institute of the Mother and Child in Warsaw, Poland).

Results: Both patients have marfanoid habitus with overgrowth, myopia, archnodactyly, pectus excavatum, hyperextensible joints, scoliosis, dysmorphic features with progeroid facial appearance associated with congenital lipodystrophy. Scaphocephalic head's shape observed especially in daughter was probably a result of wide fontanelles and disturbances in cranial sutures fusion in childhood. Mental and motor development in both were within normal limits.

Conclusions: This is the second report presenting the patient with MPLS and cranial suture development disturbances. This syndrome should be included in the differential diagnosis of Marfan syndrome and progeroid-lipodystrophy syndromes coexisting with disturbances in cranial suture development.

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P04.41A

Identification and characterization of microRNA-149, a candidate for orofacial clefting

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Introduction: Nonsyndromic cleft lip with/without cleft palate (nsCL/P) is a common facial malformation with multifactorial etiology. The majority of genetic risk loci map to non-coding regions of the genome, suggesting that the underlying pathomechanisms act through regulatory effects on gene expression. One potential mechanism might be posttranscriptional gene regulation by micro RNAs (miRNA). For nsCL/P, a suitable cellular model are human neural crest cells (hNCCs), the mesenchymal precursor cells that give rise to the majority of cranial cartilage and bones.

Methods and Results: We combined array-based miRNA profiling in hNCC with in-house GWAS data to identify candidate miRNAs for nsCL/P. This analysis revealed miR-149-3p as strong candidate for nsCL/P involvement, based on consistent hNCC-expression across replicates and the presence of associated risk variants within its genomic region. Next, we aimed at characterizing the molecular mechanisms of miR-149-3p in the context of craniofacial development. *In vitro*, we modified miRNA149 abundance levels in cultured hNCC by overexpression and inhibition assays and monitored the migration behavior of hNCC using scratch assays. We found that inhibition of miR-149-3p significantly increases cellular migration of hNCC compared to untreated hNCCs. RNA-Seq data at different time points revealed differential expression of GPC1 and BMP7. To follow up these findings, *in vivo* analyses are currently performed in the zebrafish.

Conclusion: Through integration of large-scale genetic data and expression patterns in relevant cell types we here identified a novel regulatory mechanism that is involved in craniofacial development and might be related to the etiology of nsCL/P.

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P04.43C

Detection of a new AluY insertion in the NF1 gene

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Introduction: Neurofibromatosis type I (OMIM# 162200) is a common autosomal dominant disorder associated with variants in the *NF1* gene. The spectrum of pathogenic variants is large, up to 30% affect splicing. Our diagnostic procedure combines analysis of genomic DNA and cDNA. By cDNA analysis we were able to detect a previously undescribed *Alu* insertion that was not detected by next generation sequencing (NGS).

Patient and Methods: A 44-year-old patient presented with café-au-lait spots, multiple subcutaneous nodules, vertebral neurofibroma, multiple iris hamartoma and choroidal nevi. DNA was first analyzed by a targeted capture-based NGS-panel (*NF1*, *NF2* and *SPRED1*) and MLPA analysis (*NF1* and *NF2*) with normal results. Heparin blood was used for cDNA analysis. Genetic analysis was performed on RNA extracted from short term lymphocyte culture; puromycine was used to prevent mRNA decay.

Result: cDNA sequencing revealed a deletion of exon 26 (r.3315_3496del, p.(Tyr1106Leufs*28); NM_001042492.2). Extended DNA-analysis of exon 26 and flanking regions by Sanger sequencing showed insertion of a truncated *AluY* transposable element in exon 26: c.3337_3338insAluY,3327_3337dupATTTATGAACC. The insertion leads to disruption of the 3' acceptor splice site in exon 26, deletion of exon 26 results in reading frame shift and premature stop codon. Retrospective analysis of NGS data indicated a loss of coverage in this specific region.

Conclusion: While NGS is a powerful method it has limitations in detection of more uncommon variants. In *NF1* genetic diagnostics, cDNA sequencing remains the gold standard for mutation detection, specifically for variants affecting splicing.

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P04.46B

Whole-exome sequencing and large-scale re-sequencing in nonsyndromic cleft lip with/without cleft palate identify novel susceptibility genes

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Non-syndromic cleft lip with or without cleft palate (nsCL/P) is a common congenital malformation and has a multifactorial etiology. To date, 40 genome-wide significant risk loci for nsCL/P have been identified, but these explain less than 40% of the genetic liability. Epidemiological observations suggest that a fraction of the unidentified heritability might be explained by rare dominant *de novo* mutations (DNMs) in genes involved in craniofacial development. If highly penetrant, such DNMs may be of relevance in a diagnostic setting and for genetic counselling.

We performed whole-exome sequencing in 50 trios (discovery cohort) and identified 33 rare protein-altering DNMs in 33 "candidate genes", two of them previously found associated with nsCL/P (*CDH1, TRMO*). Of note, this is the first study in nsCL/P trios using DNMs for candidate gene identification.

The "candidate genes" were subjected to resequencing with single molecule molecular inversion probes in 1,010 nsCL/P patients from European, Arabian, and Mexican ancestry and 1,574 population-matched controls (replication cohort). Of the total callset of 2,956 variants, 373 were absent from controls and showed CADD≥15 and MAF≤0.1% in public reference datasets. As a first step, we performed segregation analysis in the European cohort. Here, we could identify further DNMs in four of the genes (*CSMD1*, *MDN1*, *ANK1*, *PAXIP1*). In those genes we also found co-segregating variants (*MDN1, CSMD1*) and one compound heterozygous index (*MDN1*). RNA sequencing datasets confirmed expression of *MDN1*, *ANK1* and *PAXIP1* in relevant embryonic mouse tissues and human neural crest cells, making those genes promising candidates for functional follow-up.

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P04.47C

Genetic distribution of early onset osteoarthritis in a series of 40 patients

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Introduction: Osteoarthritis (OA) is the most common joint disease worldwide. OA is a highly heterogeneous condition, with a wide range age of onset. Non-syndromic early-onset OA (EO-OA) is very rare and mainly familial. Strict inclusion criteria for EO-OA are based on: XRays evidence, body mass index (BMI) ≤ 30, age of onset ≤ 40y, ≥ 1 joint site involved and positive familial history. Although multifactorial mode of inheritance is common in OA, EO-OA are mainly monogenic conditions. Based on these data, we aimed to study the monogenic causes of non-syndromic EO-OA in French patients.

Materials and Methods: From 2013 to 2018, clinician experts in the field of skeletal dysplasia referred EO-OA patients to our Montpellier competence center, which assessed the diagnostic and excluded chondrodysplasia, based on clinical and radiological data. For genetic analysis, we used either Sanger sequencing or an NGS custom panel approach.

Results: We recruited 40 EO-OA patients, with a mean age of onset of articular pain at 23 years, ranging from 7 to 60 years. Pathogenic or a likely pathogenic heterozygous mutations were identified in 14/40 cases (35%), with 11/14 *COL2A1* mutation (78.6%). We also observed a genetic heterogeneity involving *COL11A2*, *COL9A3*, and surprisingly a homozygous pathogenic variation in *SLC26A2*. Familial segregation of *COL2A1* pathogenic variants was observed for 6 cases.

Conclusions: We confirmed that *COL2A1* is the most common genetic cause of familial EO-OA. However, at least three other genes are involve in EO-OA. Therefore, we recommend to screen genes involved in cartilage matrix and homeostasis.

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P04.48D

Mutations in osteoarthritis susceptibility genes cause joint shape variation detectable during ontogeny in zebrafish

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Osteoarthritis (OA) is a joint degenerative disease and leading cause of pain and disability worldwide. Joint shape is commonly used to predict OA. GWAs have rapidly increased the number of OA associated genes. Despite few genes were linked to shape variation, evidence for causality and functionality or their effect on joint shape are still lacking. Therefore, there is an unmet need to develop rapid and alternative screening platforms to test OA genes. We use the zebrafish jaw joint (JJ) to investigate the impact of OA genes and ageing on joint shape variation. 3D morphometrics showed shape abnormalities in aged JJ, accompanied by OA histopathological signs. Interestingly, such modifications were prematurely found in OA mutants (*chsy1*, *col9a1*, *col11a2*, and *wnt16*). We investigated if shape changes would be detected during JJ ontogeny by testing a broader list of OA mutants (*chsy1*, *col9a1*, *col11a2*, *gdf5*, *barx1*, *mcf2l*, *dot1l*, *wnt16* and *ncoa3*) using confocal imaging of larvae immunostained for collagen type 2 followed by 2D and 3D analysis. Distinct classes of OA proteins leaded to significant shape variation in larvae, mostly explained by abnormal cell behaviour and collagen distribution. Dramatic shape variation caused joint movement impairment. We tested whether the use of mosaics, CRISPR G0s, could be sufficient to detect shape and cell changes. Surprisingly, wnt16 mosaics and *wnt16-/-* displayed similar cellular and shape changes in larvae and adults. By developing computational tools to facilitate 3D analysis we delivered a powerful and rapid screening platform to test OA genes. Versus Arthritis (grants 19497, 21161)

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P04.49A
Genetic heterogeneity in Polish patients with Osteogenesis Imperfecta

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Introduction: Osteogenesis Imperfecta is a rare genetic disorder of connective tissue characterized by numerous fractures, blue sclera, tooth abnormalities, short stature and skeletal deformity. The diversity of clinical features and its severity varies between patients, even within a single family, ranging from severe perinatal lethal to a mild form. The aim of this project was to determine genetic heterogeneity versus clinical variability observed in patients affected by OI.

Materials and Methods: NGS using custom panel of 34 genes with confirmed and probable significance in OI pathogenesis was performed. The study included 118 patients, aged 1-44 years, presenting a broad spectrum of clinical manifestation. Based on bioinformatic analysis, selected variants were verified with Sanger sequencing in a group of 77 patients.

Results: We have identified *COL1A1* mutations in 47 patients, *COL1A2* in 24 patients and mutations in 3 non-collagenous genes in 6 patients. A total of 41 different point mutations in *COL1A1* and 20 in *COL1A2* were found. Respectively 15 and 11 have not been reported in dedicated Osteogenesis Imperfecta Variant Database. We also reported patient with a deletion spanning 25 exons in *COL1A1* gene. Thus far expected lethality of particular domains in collagen type I genes revealed to be dubious, as we identified 11 patients aged 1,5 to 38 years with pathogenic mutations situated in these regions.

Conclusions: Inter- and intrafamilial phenotype variability in OI makes genetic counseling based on clinical symptoms challenging and proves panel targeted resequencing is a powerful and useful diagnostic tool. Young Scientist Grant 2016/IV/57-MN

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Genotype and phenotype in 201 Portuguese patients with osteogenesis imperfecta - unusual molecular results challenge genetic counseling

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Introduction: Osteogenesis imperfecta (OI) is a rare genetic bone fragility disorder. Although mutations in *COL1A1/2* account for 90% of cases, up to 17 different genes were associated with OI. Our aim was to characterize the clinical and mutational spectrum of OI in Portugal and to correlate genotype and phenotype.

Materials and Methods: Clinical data of 201 OI individuals (135 adults, 58 children, and 8 fetuses) from 157 families were collected through clinical evaluation and/or medical records analysis. Sanger and/or different NGS based strategies and MLPA (*COL1A1/2*) were used for molecular analysis.

Results: One hundred thirty-seven individuals had mild, 31 moderate, 24 severe, and 9 extremely severe OI. A total of 123 different variants (65 novel) were identified. Variants in *COL1A1* (n=91, including one multi-exonic deletion) and *COL1A2* (n=29, including one homozygous case) account for 85.1% of all molecularly diagnosed families (66 with quantitative and 48 with qualitative variants), followed by *SERPINF1* (n=4), *LRP5* (n=4), *IFITM5* (n=3), *FKBP10* (n=3), *WNT1* (n=2), *CRTAP* (n=1), *TMEM38B* (n=1), *P3H1* (n=1), *PPIB* (n=1), and *BMP1* (n=1). Interestingly, heterozygous variants in severe autosomal recessive OI genes were found in 3 patients with mild OI.

Conclusions: This is the first large-scale study on OI in Portugal. In accordance with other populations, quantitative variants predominate in mild *COL1A1/2* cases, and *IFITM5* and *FKBP10*-related cases had recognizable phenotypes. The identification of a homozygous variant in *COL1A2* and heterozygous variants in autosomal recessive genes complicate genetic counselling. No variant was identified in 6.5% of probands.

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P04.51C
Next generation sequencing performance in osteogenesis imperfecta

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Osteogenesis imperfecta (OI) is a connective tissue disorder characterized by bone fragility. An important clinical heterogeneity exists ranging from severe antenatal letal form to mild postnatal affection. For now, 19 genes are known to be responsible for OI: 3 dominant autosomal forms (COL1A1, COL1A2 and IFITM5), 14 recessive autosomal forms and 2 X-linked forms (PLS3, MBTPS2). Importance of a proper molecular diagnosis for genetic counseling led us to design a panel of targeted resequencing of the 19 gene coding regions, substituting for the former Sanger analyses. Some genes implicated in differential diagnoses like hypophosphatasia or overlapping phenotypes were added, increasing to 39 the total number of genes. All requests were submitted to the Reference Center for Constitutional Bone Diseases with clinical and radiological details. Libraries were prepared using capture technology (SureSelectXT Custom - Agilent) and medium throughput sequencing on a NextSEq sequencer (Illumina). Since 2015, 368 mutations were identified in 517 patients (71%): 187 in the COL1A1 gene (50%), 98 in the COL1A2 gene (26%),

15 in IFITM5 (4%), 48 in recessive genes responsible for OI (13%), 3 in X-linked gene. All these mutations were confirmed by Sanger sequencing. The results are discussed during a feedback meeting with clinicians and biologists. This panel allows efficient screening for all OI genes, leading to time and cost-savings. Using a panel strategy avoid incidental findings. The main limit is the occasional difficulty to determine the pathogenicity of the identified variant. Whole exome sequencing analysis can be proposed for patient without mutation.

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Diagnostic yield of NGS analysis of a panel of Osteogenesis imperfecta-related genes in 550 patients with Osteogenesis imperfecta, (early-onset) nonsyndromic osteoporosis and related disorders

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Introduction: Our Genomediagnostics laboratory in Amsterdam UMC offers targeted NGS analysis of genes involved in Osteogenesis imperfecta (OI) and other connective tissue disorders. OI is characterized by bone fragility and fractures, bone deformities, short stature, dentinogenesis imperfecta, hearing loss, and blue sclerae. The phenotype is highly variable, from mild to perinatal lethal. Osteoporosis is a common, multifactorial disorder, characterized by reduced bone-mass and fractures. A few genes have been associated with (early-onset) monogenetic non-syndromic osteoporosis (OP). Here we present the results of the analysis of a panel of 19 OI/OP-related genes in 550 patients referred with OI, OP or a related disorder.

Material and Methods: A solution-based enrichment kit was designed to capture exons and splice sites of the target genes. Data were analysed using an in-house pipeline and Cartagenia, and an NGS-based CNV analysis tool. 68% of the patients (mean age 37y) had osteoporosis and/or fractures; no additional OI-related features were reported (OP-cohort). 23% of the patients (mean age 22y) had at least one other OI-related symptom reported (OI-cohort). **Results** Diagnosis was molecularly confirmed in 67% of the patients in the OI-cohort, versus 6% in the OP-cohort. In the OP-cohort, a suspicious VUS was identified in 12% of the patients. Variant distribution among genes was different between OI- and OP-cohorts.

Conclusions: As expected, the yield of genetic testing was significantly higher in the OI-cohort versus the OP-cohort. However, the proportion of patients with a (potential) genetic cause in the OP-cohort is relevant and warrants further investigation of these patients.

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P04.53A

Between LSD and skeletal dysplasia - a rare case of Touraine-Solente-Golé syndrome caused by new mutation

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Introduction: Primary hypertrophic osteoarthropathy (PHO), also known as pachydermoperiostosis (PDP) or Touraine-Solente-Golé syndrome, is a rare genetic osteo-arthro-dermopathic syndrome with familial and idiopathic forms differentiating it from secondary (pulmonary) hypertrophic osteoarthropathy. Though the diagnosis can be made on the basis of the classic clinical and radiological findings, it is often missed due to variable presentations PHO is characterized by digital clubbing, periostosis, acroosteolysis, painful joint enlargement, and skin manifestations that include thickened facial skin, a thickened scalp, and coarse facial features. The disorder has estimated prevalence of 0.16%, and usually manifests in adolescence, occurring almost exclusively in males, with a M: F ratio of 7:1.

Materials and Methods: We present a 15 years old boy, from Afghanistan, born to consanguine parents who presented for the first time in our department with painful swelling of hands, feet, and face, coarse facial features, hypertrichosis, short and broad palms, and soles. He reports on elder brother with similar swelling, and alopecia. Radiological examinations show wide and thick metacarpals, short distal phalanges, square-like vertebras.

Results: Many tests were performed to rule out lysosomal storage disorders, and skeletal dysplasia. After performing an NGS-analysis (clinical exome sequencing), the patient was found to carry, in homozygous state, a new mutation (c.290G>A) in *SLCO2A1* gene causing PHO type 2.

Conclusions: PDP type 2 has diverse radiological and clinical features. It may remain undiagnosed for long time and progress until there are significant facial, joint, and digital deformities that finally make the patient seek medical attention.

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P04.54B
Dental Management Experience for Patients Suffering from Papillon-Lefévre Syndrome

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Introduction: Papillon–Lefévre syndrome (PLS) is a rare autosomal recessive disorder caused by mutation of the Cathepsin C gene. Patients mainly suffer from palmoplantar keratosis, periodontitis, and early loss of teeth. The present study is concerned with the dental management of PLS patients through periodontal and prosthetic intervention.

Materials and Methods: Nine PLS patients were included in this study. The patients were referred to our Orodental Genetics Clinic complaining of early loss of the deciduous dentition and were in different stages of permanent teeth eruption. Our management protocol included; conventional scaling and root planning every 3 month, diode laser curettage and pocket sterilization monthly sessions and construction of a removable prosthesis to restore the lost teeth. Pocket depth, bleeding index and mobility was assessed every 6 month during the 2-year period of the study.

Results: All patients showed good response to the laser treatment. Gingival inflammation was controlled with less bleeding, decreased mobility of teeth and no teeth were lost over 2 years. However, panoramic radiographs showed bone loss in the pocket areas.

Conclusion: Laser pocket disinfection was effective in reducing gingival tissue inflammation, yet the progressive bone loss was a concerning outcome that needs further investigations.

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P04.55C
Abnormal activation of mutated complement 1 subunits C1r/C1s in periodontal Ehlers-Danlos syndrome

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Introduction: Periodontal Ehlers–Danlos syndrome (pEDS) is a connective-tissue disorder characterized by early severe periodontitis and various joint and skin manifestations. Most EDS types are caused by mutations in collagens or protein-modifying enzymes. In contrast, periodontal type is caused by heterozygous missense or in-frame insertion/deletion mutations in *C1R* or *C1S*, indicating a previously unknown connection between the inflammatory complement pathway and connective tissue homeostasis. pEDS is autosomal dominant and involves gain-of-function effects; loss-of-function variants in *C1R/C1S* are asymptomatic when heterozygous and can cause a lupus-like phenotype when homozygous.

Methods and Results: In-vitro overexpression in HEK293T cells demonstrated that all pathogenic *C1R*

variants except a mutant in the C1q binding motif cause abnormal intracellular processing and secretion of enzymatically active serine protease. Unlike C1r wild type, mutations in the CUB1 and CCP1 domains of C1r show intracellular retention of the N-terminal fragment. Mutations in the CUB2 domain cause secretion of aggregated N-terminal fragments, whereas CCP2 domain mutations induce a new cleavage site. Importantly, the C-terminal catalytic fragment from all C1r mutants was secreted and enzymatically active in the supernatant. Western blot analysis of patient-derived skin and gingival fibroblasts confirmed aberrant activation and secretion of mutated C1r even in the presence of C1s and C1 inhibitor. C1s activation was confirmed by *in-vitro* complement activation assays.

Conclusion: pEDS is caused by gain-of-function mutations that cause abnormal activation of complement 1 independent of microbial triggers. We hypothesize that secreted catalytic fragments cleave extracellular matrix proteins with adverse consequences on connective tissue homeostasis.

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P04.57A
Genotype of Bruck syndrome with phenotype of Osteogenesis Imperfecta, separate syndromes or expansion of the spectrum

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Introduction: Osteogenesis Imperfecta (OI) is heterogenous group of disorders with increased bone fragility. Bruck Syndrome (BS) is a rare autosomal recessive syndrome characterized by OI with congenital contractures due to mutations in *FKPB10* and *PLOD2* genes (BS1 and BS2 respectively). *FKPB10* mutations were found to cause moderately severe OI type XI and PLOD2 was reported before to cause fractures without contractures. Herein we present two patients from two Egyptian families with recurrent fractures but without joint contractures having *PLOD2* mutations.

Materials and Methods: two unrelated Egyptian consanguineous families with repeated fractures were studied. Mutation analysis was performed using NGS panel, homozygosity mapping, then candidate gene approach.

Results: the first family included a 15 years male who presented with repeated fractures around 10 times/year, deformities and severe kyphoscoliosis (clinical severity score; CSS 17). The second family had a 9 years old girl milder in severity with 1-2 fractures/year (CSS 14). Both patients had no joint contractures. They were short and had Wormian bones in their skull x-rays and osteoporosis confirmed by DEXA. Molecular testing for both patients revealed homozygous donor splice site mutation in *PLOD2* in intron 12(c.1358+5G>A). The mutation was heterozygous in the parents.

Conclusion: This study and previous reports from other centers identified different *PLOD2* mutations with repeated fractures and variable severity but without contractures and this expands the phenotypic spectrum of PLOD2 associated mutations. Accordingly, it's important to reconsider the current classification to include BS as a variant of OI subtypes rather than being a separate syndrome.

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P04.58B
Clinical and molecular characteristics of *GNAS* inactivation disorders observed in 18 Korean patients

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The *GNAS* gene on chromosome 20q13.3 is a complex, imprinted locus regulated in a tissue-specific manner. *GNAS* inactivation disorders are a heterogeneous group of rare disorders caused by mutations and methylation defects. These are divided into pseudohypoparathyroidism (PHP) types 1a and 1b, pseudo-pseudohypoparathyroidism (PPHP), and progressive osseous heteroplasia (POH), depending on the presence or absence of hormone resistance, Albright's hereditary osteodystrophy (AHO), and ectopic ossification. This study analyzed the clinical characteristics and molecular genetic backgrounds of 18 Korean patients from 16 families with a genetically confirmed *GNAS* defect. Auxological parameters, AHO phenotypes, types of hormonal resistance, family history, and molecular genetic disturbances were reviewed retrospectively. Nine (90%) patients with PHP1a showed resistance to parathyroid hormone (PTH) and all patients showed elevated thyroid-stimulating hormone (TSH) levels at diagnosis. Eight (80%) patients were managed with levothyroxine supplementation. Three of six patients with PHP1b had elevated TSH levels, but none of whom needed

levothyroxine medication. AHO features were absent in PHP1b. Patients with PPHP and POH did not show any hormone resistance. Among the 11 families with PHP1a, PPHP, and POH, eight different (three novel) mutations in the *GNAS* gene were identified. Among the six patients with PHP1b, two were sporadic cases and four were familial cases. This, the largest single-center series study of *GNAS* inactivation disorders in Korea, summarizes the detailed clinical and molecular genetic characteristics of these diseases. Identification of molecular genetic backgrounds, along with clinical phenotypes, enables appropriate management and proper genetic counseling.

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P04.59C
Compound screening for PXE using zebrafish *abcc6a* mutant models: a proof-of-concept study

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Introduction: Pseudoxanthoma elasticum (PXE) is an ectopic mineralization disease due to biallelic *ABCC6* mutations. As no curative therapy is available, we characterized a zebrafish *abcc6a* CRISPR/Cas9 knockout model (*Cmg52*) for drug screening purposes, identifying spinal hypermineralization as an early, quantifiable phenotype. As a proof-of-concept we tested our screening workflow on two compounds (80μM Vitamin K1 [VK1] and 30μM Sodium Thiosulfate [STS]), both implicated in PXE.

Materials and Methods: Following $Cmg52^{+/-}$ incross, $Cmg52^{+/+}$, $Cmg52^{+/-}$and $Cmg52^{-/-}$ embryos were collected. At 3 days post-fertilization (dpf), embryos are distributed per 20 in baskets. From 3-10dpf, 60 embryos are treated with 8ml compound in 1x E3-medium and refreshed daily. Following euthanasia at 10dpf, embryos are fixed, bleached and stained for mineralization. Photos are taken of embryos under identical conditions and via ImageJ mineralization is quantified. DNA is extracted from embryos and mineralization values are linked to respective genotypes for statistical analysis.

Results: VK1 significantly reduced spinal mineralization in $Cmg52^{-/-}$ ([Mean ± SD] Controls: 19.371 ± 12.794; VK1: 10.322 ± 5.519; $P<0.05$). STS-treated larvae had no spinal mineralization or higher mortality compared to controls. However, 33% of STS-treated animals had spotty abdominal mineralization regardless of genotype, indicating putative toxicity.

Conclusions: We confirmed the $Cmg52^{-/-}$ mineralization phenotype to be a good read-out for compound screening. Our data suggests a role for VK1 in PXE pathogenesis though further validation is required. The STS data underscores the importance of proper controls and that compound screening data need to be interpreted cautiously.

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P04.61A
Retinoic acid catabolism defects affecting skeletogenesis and resembling craniosynostosis syndromes

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Introduction: Homozygous missense mutations in the *CYP26B1* gene, have been described in very few individuals with phenotypes ranging from a lethal generalised skeletal dysplasia characterised by severe skull defects and craniosynostosis, radiohumeral fusions, oligodactyly and narrow thorax to a milder presentation described in one adult affected by multiple craniosynostosis, characteristic facies, radiohumeral joint limitation, hearing loss and intellectual disability. The enzyme coded by *CYP26B1* is involved in retinoic acid catabolism and regulates its precise temporo-spatial gradient during embryogenesis. Case presentation: We describe a healthy non-consanguineous couple with 2 children with distinctive craniofacial features including turribrachycephaly, shallow orbits, malar hypoplasia, low-set and protruding ears, small mouth, high palate with crowding teeth and pointy chin. Both siblings have bilateral conductive hearing loss, arachnodactyly, reduced movement of the radio-ulnar joints and mild to moderate learning disability. Their phenotype resembles that of the reported adult.

Results: Whole exome sequencing revealed both sibs inherited two missense mutations in trans affecting *CYP26B1*. Variant c.353C>T (p.P118L) was paternally inherited, not previously described and not seen in large population cohorts, and variant c.701G>A (p.R234Q), with frequency of 0.06% in large databases, was maternally inherited. Both variants were predicted to have a deleterious impact on the protein structure.

Conclusion: Our patients add to the wide phenotypic constellation seen with *CYP26B1* pathogenic variants. We infer that higher retained enzyme activity is non-lethal and

mostly associated with skeletal and neurological features including characteristic craniofacial features resembling craniosynostosis, hearing loss and intellectual disability. Funding source: Rare disease Foundation microgrant. Year 2018 #2803.

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P04.62B

The splicing variant c.1815G>A in *KIAA0586* is associated with a phenotype overlapping short rib polydactyly and oral facial digital syndrome

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Ciliopathies are a group of hereditary disorders caused by abnormal structure/function of primary cilia, which are ubiquitously expressed organelles characterized by a mother centriole-derived basal body, a microtubule-based axoneme and a specialized membrane harboring proteins required for signal detection and amplification. Causative variants in *KIAA0586*, the human ortholog of chicken talpid3 essential for primary ciliogenesis and hedgehog signaling, were first associated with Joubert syndrome, a multisystem disorder displaying pathognomonic hindbrain malformation as well as variable skeletal, renal and ocular defects. *KIAA0586* is also associated with other ciliopathies such as the short rib-polydactyly, a lethal bone dysplasia with severely hypoplastic thorax. Here we describe two Roma Gypsy siblings affected by a neonatal lethal short rib-thoracic dysplasia, harboring the *KIAA0586* homozygous variant c.1815G>A (p.Gln605Gln), identified by targeted resequencing analysis. Clinical features of this family are not limited to the typical short rib-polydactyly syndrome but also include tongue/oral hamartomas, multiple frenulae and cleft palate. The same combination of intraoral findings have been already identified in patients with the esonic splice site variant p.Gln605Gln; suggesting continuity between short-rib-polydactyly and oral-facial-digital syndromes in the context of *KIAA0586* clinical spectrum.

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P04.63C

Chipping away the challenges of diagnostic genomics applications for short stature

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Introduction: As for many common complex disorders, diagnostic genomics applications for short stature (SS) face many challenges. In the absence of consensus international guidelines on genetic testing for SS, various clinical criteria are used to inform appropriate testing. The extent of genetic heterogeneity and complexity of SS are still being elucidated, challenging diagnostic approaches.

Materials and Methods: We employed a retrospective analysis of diagnostic genomics yield for SS patients receiving growth hormone therapy on the Western Australian Paediatric Endocrine Database. In addition, a prospective systematic study using chromosomal microarray and a clinical exome panel on cases with unknown genetic aetiology was initiated in 2018.

Results: A major genetic aetiology was identified in ~31% of cases prior to the systematic application of contemporary genomic technologies (35 out of 114 participants born since 2005 and receiving growth hormone for >2 years). Presence of composite genomic findings in some patients challenged interpretation. Referral to a local interdisciplinary undiagnosed disease program helped in resolving the diagnostic odyssey for complex patients.

Conclusions: Diagnostic genomics applications for SS are still evolving and some of the challenges can be overcome by pre-test clinical selection, detailed phenotyping and contribution of an inter-disciplinary team input. The current efforts focus on the detection of high penetrance genetic causes, and the remaining challenge is to interpret

moderate to low penetrance genetic risk factors for SS. Acknowledgements: Merck Serono Australia, grant 201603.4701.POT; DNA is supported by a Raine clinical research fellowship (CRF018, project "Diagnostic genomics applications for short stature").

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P04.64D
Identifications of novel variants affecting *SHOX* expression in Short Stature patients

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Haploinsufficiency of the pseudoautosomal *SHOX* gene causes 70-90% of Léri-Weill dyschondrosteosis (LWD) and 2-10% of idiopathic short stature (ISS). Deletions removing the gene or enhancers and nonsense/frameshift mutations represent a well-established cause of disease. Otherwise, rearrangements not encompassing any described enhancer and 5'UTR variations are also identified in patients but their pathogenic role remains unclear. During diagnostic screening performed on 934 patients by standard methods (MLPA and sequencing) we identified *SHOX* alterations in 82 patients (8.8%). Among these, 65 (78%) carried deletions while 17 (22%) point mutations. Besides these pathogenic mutations we also identified small deletions not encompassing any already described enhancer (n=11) and variations within 5'UTR (n=5) in 24 LWD/ISS patients whose pathogenic role was less clear that were classified as VOUS. The aim of this work was to investigate through functional studies the pathogenic role of these variations. To better characterize the deletions not encompassing the enhancers we used a fine-tiling custom aCGH and performed *in vitro* functional assays to assess the potential enhancer activity of conserved sequences within the deleted areas. The variants within the 5'UTR were tested for their ability to interfere with correct gene expression. Our results showed that some of the VOUS might be responsible of *SHOX* deficiency either by removing putative regulatory elements or affecting the proximal promoter activity. In conclusion, the present study allowed us to reclassify as likely pathogenic some of the novel variants of uncertain significance, that we detected in the diagnostic screening, thus reducing them of at least 10%.

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P04.66B
Report of a novel variant causing a schneckenbecken-like dysplasia

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Introduction: A homozygous pathogenic variant in *SLC35D1*, an endoplasmic reticulum nucleotide sugar transporter, was identified as a cause of schneckenbecken dysplasia in 2007. We report a case of a skeletal dysplasia with inconclusive pathological and radiological features and a missense change in *SLC35D1*.

Material and Methods: A consanguineous family had 4 pregnancies affected by a skeletal dysplasia. Insufficient DNA from the 4 pregnancies meant that exome couple analysis was undertaken on the parents followed by in silico protein modelling using Phyre2 web and I-TASSER servers. Functional effects of variants were assessed using a proteo-liposome assay (Rautengarten et al., 2014 PNAS 111: 11563-11568).

Results: Both parents were heterozygous for a missense change in *SLC35D1* c.398C>T p.(Pro133Leu). Reappraisal of the radiographs was consistent with schneckenbecken-like dysplasia. Testing of one of the fetuses and 2 healthy unaffected siblings was consistent with autosomal recessive inheritance but not sufficient to confirm pathogenicity. In silico protein modelling showed that p.Pro133 lies directly opposite p.Thr65, the site of the only previously reported

pathogenic missense variant, at the mouth of the solute channel, implying pathogenicity. The prote-liposome assay demonstrated 2-4% in transport activity compared to wild-type but also showed *SCLD231* is a general UDP-sugar transporter with clear activity towards UDP-Xylose, suggesting a mechanism by which the Golgi synthesized substrate can be imported into the endoplasmic reticulum for essential xylosylation reactions.

Conclusion: An agnostic approach to molecular testing together with protein modelling and a functional assay demonstrated pathogenicity of an *SLC35D1* variant and increased the protein's biochemical characterization.

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P04.67C

A novel *POC1A* variant in exon 2 causes classical SOFT syndrome: Clinical presentations of seven patients

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Introduction: Biallelic pathogenic variants in *POC1A* (centriolar protein A) cause either SOFT (Short-stature-Onychodysplasia-Facial-dysmorphism-and-hypoTrichosis) syndrome or a milder and only partially overlapping phenotype, i.e., variant *POC1A*-related syndrome – with this pleiotropic effect depending on the respective exon affected by mutation. Pathogenic changes in a total of only 22 patients had been described underlining the disease rareness. Two large consanguineous families of Omani origin presented to the clinic with 7 individuals affected by primordial dwarfism resembling SOFT syndrome, but of unknown genetic cause and diagnosis.

Materials and Methods: Detailed clinical work-up to define the symptoms and whole-exome sequencing (WES) were performed in one of the index patients and two relatives, as well as subsequent Sanger sequencing for further segregation studies.

Result: WES yielded the variant *POC1A* (NM_015426.4): c.64G>T p.(Val22Phe) which showed segregation within the tested individuals and further family members. This variant affects the alternative exon 2, that is present only in two of three *POC1A* transcripts. Nevertheless, all of the investigated patients had comparable disease histories with severe growth retardation of prenatal onset, characteristic facial features and respiratory difficulties, the latter being most prominent in the index patient; which confirmed the diagnosis of SOFT syndrome.

Conclusion: The finding i) considerably increases the number of reported SOFT syndrome patients allowing for further clinical delineation of this rare disorder, and ii) add a novel *POC1A* missense variant in exon 2, allowing to refine the relevance of the functional domain affected by this change and expand the genotype- phenotype correlation in *POC1A*-related disorders.

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P04.69A

Phenotypic overlap between spondyloepimetaphyseal dysplasia with joint laxity type 2 and Morquio syndrome type A: case report

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Introduction: We present fifteen-year-old boy patient, with complex skeletal phenotype highly susceptible to mucoplysaccharidosis type IVA (Morquio syndrome). He has severe skeletal dysplasia including disproportionate short stature with short trunk, kyphoscoliosis with gibbus, joint hypermobility, bilateral congenital hip dislocations, knock-knee and ulnar deviation of the wrists. The patient is immobile since his sixth year. He doesn't have hepatosplenomegaly, respiratory complications, cardiac valve abnormalities and dental abnormalities. No pathological ophthalmology findings, neither hearing loss.

Materials and Methods: We performed enzymes blood testing for several types of mucopolysaccharidosis in first line testing, and exome sequencing which targeted genes

related to observed clinical presentation in second line testing.

Results: Unexpectedly, enzyme level of N-acetylgalatosamine-6-sulfate-sulphatease showed normal result and excluded Morquio syndrome. Subsequently, exome sequencing showed presence of heterozygous pathogenic missense variant in *KIF22* gene (c.443C>T, p.Pro148Leu). Pathogenic variants in *KIF22* gene represent an established cause of spondyloepimetaphyseal dysplasia with joint laxity type 2 (OMIM:603546). The clinical presentation of KIF22-associated conditions includes skeletal abnormalities, joint laxity, joint dislocations, spinal deformities and short stature, which is compatible with the clinical presentation in our patient.

Conclusion: Between Morquio syndrome and spondyloepimetaphyseal dysplasia may exist completely overlapping skeletal phenotype, which is confirmed literature data (ref. Biswas SN et al., 2017). The distinction between these diseases is very important because of available enzyme replacement therapy for Morquio syndrome type A and completely different pattern of inheritance and recurrence risk. Exome sequencing should be first genetic test in patient with severe skeletal dysplasia with overlapping phenotype.

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P04.70B
Further evidence for COL9A3 associated autosomal recessive Stickler syndrome

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Heterozygous COL9A3 [MIM 120270] mutations were reported to cause multiple epiphyseal dysplasia-3 [MIM 600969] which is mainly characterized by stiffness and/or pain in the knees, due to flattened, irregular epiphyses, and varus or valgus deformity, waddling gait, and mild short stature in some patients. Here we report on the second case with bi-allelic COL9A3 nonsense mutations. The boy was born with club feet and moderate hearing loss to healthy parents. Radiographs revealed flat lumbar vertebrae, coxa retrotorta, and broad femoral metaphyses. At examination at age 2,5 years we saw a triangular face, bilaterally mild epicanthus, a high arched palate, and short fingers. In addition, myopia was diagnosed recently. Height was 92 cm, weight 15 kg (50. and 75. centile, respectively). Because of the phenotype, the provisional diagnosis of Stickler syndrome was made and molecular analyses revealed compound-heterozygosity for COL9A3 mutations c.268C>T; p.(Arg90*) and c.1739dup; p.(Gly581Trpfs*20). Bi-allelic COL9A1 [MIM 120210] and COL9A2 [MIM 120260] mutations are already known to cause autosomal-recessive inherited Stickler syndrome [MIM 614134; 614284]. However, as far as we know, only one family with bi-allelic occurrence of a COL9A3 nonsense mutation and Stickler syndrome is published until now. As in our patient, in this family the affected individuals showed a comparatively mild phenotype with no vitreoretinal involvement or cataracts so far and no cleft palate/Pierre-Robin sequence. Thus, the findings in our case further support the assumption that bi-allelic COL9A3 mutations are very likely sufficient to cause Stickler syndrome as well.

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P04.71C
Stüve-Wiedemann syndrome: a rare clinical entity

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Introduction: Stüve-Wiedemann syndrome(SWS)(MIM601559) is an autosomal recessive skeletal dysplasia characterized by bowing of extremities, severe osteoporosis, joint contractures, dysautonomia, frequent respiratory infections, and feeding difficulty. Mutations in *LIFR* are responsible for the syndrome. Although the majority of patients are lost due to complications in infancy, patients who reach adolescent period are reported rarely. Here, we present a 9 years-old male with SWS with severe skeletal findings due to a novel homozygous mutation in *LIFR*.

Materials and Methods: The patient was the third child of healthy, consanguineous parents, born at term with a birth weight of 3600g(50-75th centile). Bowing in lower extremities was remarkable at birth, and progressed gradually with age. He was hospitalized many times for respiratory distress, feeding difficulties, and recurrent fever. Motor developmental milestones were delayed however, mental development was normal. Physical examination revealed facial dysmorphic features, bending of the extremities, laxity of the metacarpophalangeal joints, scoliosis, prominent heels, and pes planus. X-ray of extremities showed bowing of the limbs, bilateral valgus deformity, and widening of the femoral and tibial metaphysis.

Results: Next-generation sequencing was performed on Illumina MiSeq(v1.9) platform using the virtual panel for skeletal dysplasia consisting of 130 genes. The novel homozygous variant in *LIFR*(c.274C>T) was detected. Segregation within the family showed that parents were heterozygous carriers.

Conclusions: Although SWS is a rare genetic disease, it should be kept in mind in patients with congenital bowing in extremities. Targeted exome analysis has great importance in the fast and accurate setting in the diagnosis of heterogeneous groups of syndromes such as bent bone dysplasias.

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P04.73A
Novel VPS33B mutation in a patient with autosomal recessive keratoderma-ichthyosis-deafness (ARKID) syndrome

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Autosomal recessive keratoderma-ichthyosis-deafness (ARKID) syndrome is a rare multisystem disorder caused by biallelic mutations in *VPS33B*; only three patients have been reported to date. ARKID syndrome is allelic to arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome (MIM #208085), a severe disorder with early lethality whose phenotypic characteristics also include ichthyosis, hearing loss, severe failure to thrive, platelet dysfunction and osteopenia. We report on an 11-year-old male patient with ARKID syndrome and compound heterozygous *VPS33B* mutations, one of which [c.1440delG; p.(Arg481Glyfs*11)] was novel. Clinical features of this patient included ichthyosis, palmoplantar keratosis, hearing loss, intellectual disability, unilateral hip dislocation, microcephaly and short stature. He also had copper hepatopathy and exocrine pancreatic insufficiency, features that have not been associated with neither ARKID nor ARC syndrome so far. The patient broadens the clinical and molecular spectrum of ARKID syndrome and contributes to genotype-phenotype associations of this rare disorder.

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P04.74B
Analysis of a French cohort of patients with Werner syndrome; clinical and molecular features

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Introduction: Werner syndrome (WS) belongs to adult onset progeroid diseases, leading to a premature ageing of mesenchymal and epithelial tissues, affecting one person per a million in the world. Its cardinal signs are short stature, lipodystrophy and atrophic skin, premature cataract and greying of hair; age-associated complications may occur in WS such as osteoporosis, chronic skin ulcers or diabetes mellitus, and patients usually die around 53 years of age from cardiovascular or neoplastic causes. This recessive genetic affection is linked to loss-of-function mutations of *WRN* gene, coding for a helicase involved in multiple DNA pathways. No recent clinical nor molecular description were available for French WS patients.

Methods: Data from French laboratories found 36 genetically confirmed WS cases, whose 34 for which molecular and clinical data were collected.

Results: 31 mutations including 14 newly described ones were identified, with some associated with a specific French region. The mean age for diagnosis was 40.5 years, the cardinal signs were found in all the patients, and under-estimated features (deafness, hepatic, thyroid, valvular, hypertensive or capillary impairments) were commonly diagnosed. Death occurred at 53.6 years of age, mostly from cancerous etiologies. Despite the homogeneity of the main signs, it seems that phenotypes with less severe evolution may exist, without associated predictive or causal factors.

Conclusion: This cohort gives an exhaustive description of French patients presenting genetically confirmed WS since the beginning of *WRN* testing. It defines accurate frequencies for all the numerous symptoms and gives the opportunity for prospective investigations.

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P04.75C
Genetic predisposition to systemic sclerosis

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Systemic sclerosis (SSc) is a multi-system disease of unknown cause, characterized by vascular and immune dysregulation that results in progressive fibrosis of the skin and other organs. While rare, it can be lethal, and there are currently no treatments. SSc is typically sporadic; familial forms are extremely rare. By applying genome-wide Next Generation Sequencing (NGS) to such families, we aim to identify genetic "drivers", i.e., disease-causative or strongly-predisposing alleles. We hypothesize that these genes may also contribute to sporadic disease, when they carry strong *de novo* mutations, weak inherited variants (that cause disease only in combination with other genetic and environmental factors), or somatic mutations in affected tissues. We performed whole exome sequencing (WES) on blood-DNA from two affected first-degree relatives each from five families, and filtered for genes with missense or nonsense variants that (i) co-segregate with disease, (ii) are rare in public sequence databases and an in-house WES database, and (iii) predicted to affect protein function *in silico*. Between 18 and 40 such genes were identified per family. Candidate gene prioritization was performed using literature and database-mining, as well as intersection with RNASeq data from sporadic SSc cutaneous tissue biopsies. Variants in the two highest-ranked candidates (one a pro-fibrotic growth factor, the other a cytokine receptor with pro-fibrotic, pro-inflammatory effects) are currently being tested for their effects on protein function. Proof of an effect on protein function will be followed by testing for a role in a bleomycin-induced mouse model of SSc.

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P04.76D
WNT10A mutations: refining genotype, phenotype, penetrance, clinical variability and inheritance manner

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Introduction: The agenesis of several teeth is a prevalent malformation in human. Hypodontia is defined by the agenesis of six teeth or less, whereas oligodontia refers to the agenesis of more than six teeth, excluding the third molars. Hypodontia and oligodontia can be associated with defects in the development of other ectodermal structures such as hairs, sweat glands and nails, defining the spectrum of ectodermal dysplasias. Mutations in *WNT10A* are known as a major cause of oligodontia with or without associated ectodermal dysplasia signs.

Patients and Methods: Based on the analysis of a large series of 413 probands with hypo or oligodontia and 190 of their relatives, we report 395 subjects with mutations in the gene *WNT10A*.

Results: We found a mutation in *WNT10A* in 227 of the 413 probands, among which two recurrent mutations the c.682T>A (p.Phe228Ile) and c.321C>A (p.Cys107Ter) are respectively involved in 178 and 33 unrelated probands and families. We show a pattern of tooth agenesis in patients with *WNT10A* mutations and a broad phenotypic variability despite a correlation between the number of mutations, the number of tooth agenesis and the presence of ectodermal signs. We confirm the incomplete penetrance of *WNT10A* mutation with an estimate of the penetrance and report several asymptomatic cases with biallelic mutations.

Discussion: Despite some degree of genotype-phenotype correlation, we highlight the incomplete penetrance and broad clinical variability associated with *WNT10A* mutations.

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P05
Cardiovascular disorders

P05.01A
European Reference Network For RareVascular Diseases (VASCERN) consensus statement for the screening and management of patients with pathogenic ACTA2 variants

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The *ACTA2* gene encodes for smooth muscle specific α-actin, a critical component of the contractile apparatus of the vascular smooth muscle cell. Pathogenic variants in the *ACTA2* gene are the most frequently encountered genetic cause of familial thoracic aortic aneurysm/dissection (FTAAD). Although TAAD is the main clinical manifestation, a variety of occlusive vascular disease and extra-vascular manifestations occur in *ACTA2* related vasculopathy. Current data suggest possible mutation-specific manifestations of vascular and extra-aortic traits. Despite its relatively high prevalence, comprehensive recommendations on the care of patients and families with pathogenic variants in *ACTA2* have not yet been established. We aimed to develop a consensus document to

provide medical guidance for all health care professionals involved in the recognition, diagnosis and treatment of patients and relatives with pathogenic variants in *ACTA2*. The hereditary thoracic aortic disease (HTAD) Working Group of the European Reference Network for Rare Vascular Diseases (VASCERN) convened to review current literature and discuss expert opinions on clinical management of *ACTA2* related vasculopathy. This consensus statement summarizes our recommendations on diagnosis, monitoring, treatment, genetic counselling and testing in patients with *ACTA2* related vasculopathy. However, there is a clear need for additional prospective multicenter studies to further define proper guidelines.

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P05.02B

Novel approach reveals celiprolol but not losartan as medical therapy for vascular Ehlers-Danlos syndrome

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Aims: Antihypertensive drugs are included in the medical therapy of vascular Ehlers-Danlos syndrome (vEDS). The β-blocker celiprolol has been suggested to prevent arterial damage in vEDS, but the underlying mechanism remains unclear. It is also unknown whether the widely-used AGTR1-antagonist losartan has a therapeutic effect in vEDS. We evaluated the impact of celiprolol and losartan on the biomechanical integrity of the vEDS thoracic aorta.

Methods: We (re-)characterised a murine vEDS model at molecular level using WGS and developed an objective approach to measure the maximum tensile force at rupture of uniaxially-stretched murine thoracic aortic rings. To assess treatment effect, heterozygous mice at 4 weeks of age underwent a 4-week treatment with celiprolol, losartan, and, as a proof-of-concept drug, the MMP-inhibitor doxycycline.

Results: Heterozygous mice showed a significant reduction in the rupture force compared to wild-type mice. Compared to age- and sex-matched untreated heterozygous mice, treatment with doxycycline or celiprolol resulted in a significant increase of rupture force, whereas no significant change was detected upon losartan treatment.

Conclusions: Our novel read-out system is suitable for the assessment of the biomechanical effect of candidate drugs. In a vEDS model, celiprolol or doxycycline, but not losartan, can improve the biomechanical integrity of the aortic wall. As doxycycline is a broad-spectrum antibiotic with considerable side effects, celiprolol may be more suitable for a long-term therapy and thus rather indicated for the medication of patients with vEDS. This is the first study demonstrating that celiprolol improves the rupture force of the thoracic aorta.

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P05.03C

Next Generation Sequencing identifies novel variants in *TJP1*, *TP63* and *PPP1R13L* genes in Arrhythmogenic Cardiomyopathy patients

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Among the most common inherited cardiomyopathies, arrhythmogenic cardiomyopathy (ACM) is characterized by progressive myocardial fibro-fatty replacement, arrhythmias and risk of sudden death. Mutations in genes encoding proteins of cardiac intercalated discs account for about 60% of ACM cases, but the remaining 40% is still genetically elusive.

Aim: We aim at identifying novel ACM genes by next generation sequencing.

Methods and Results: The DNA of 40 ACM patients was analyzed using a targeted gene panel consisting of 15 known
ACM genes and 53 candidate genes. We found two novel variants in *TP63 (c.796C>T, p*.R266*) and *PPP1R13L* (c.1858G>C, p.A620P) candidate genes, encoding for the proteins p63 and iASPP, respectively. The *TP63* variant is scored as likely pathogenic and the *PPP1R13L* variant as of uncertain significance and involves a conserved functional domain.
Importantly, the mutant *TP63* allele leads to nonsense-mediated-mRNA decay, causing
haploinsufficiency.

Also, novel variants in *TJP1* gene were detected by whole exome sequencing in additional 4 ACM Italian and Dutch/German probands. Out of the 4 variants, p.Y669C in the encoded protein ZO-1 promotes structural rearrangements of the GUK domain, whereas the p.R265W, p.S329L, and p.D360V are predicted to impair the function of the involved the disordered region between PDZ2 and PDZ3 domains. Furthermore, rare variants in *TJP1* are significantly enriched in patients with ACM relative to controls.

Conclusions: We provide the first evidence linking *TP63* and *TJP1* variants to ACM, while the functional involvement of *PPP1R13L* remains to be determined.

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P05.04D

Aortic dilatation in patients with Traboulsi syndrome

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Traboulsi syndrome (OMIM 601552) is a rare autosomal recessive disorder caused by mutations in ASPH (OMIM 600582). It is characterized by ectopia lentis, spontaneous filtering blebs, other anterior segment anomalies and craniofacial dysmorphism. Thirteen patients have been reported, and aortic dilatation has not been noted, though details regarding aortic measurements have not been consistently provided. We report three paediatric patients from two families affected with Traboulsi syndrome presenting with mild-moderate aortic dilatation in addition to the ocular phenotype. The two affected individuals in Family 1 have dilation of the aortic root (Patient 1-I aortic sinus 30.2 Boston z-score 2.2, Patient 1-II aortic sinus 28.2 mm, Boston z-score 4.1, and sinotubular junction 23.7 mm, Boston z-score 4.7). In addition Patient 1-I has a myxomatous mitral valve with regurgitation, and Patient 1-II has a bicuspid aortic valve. Both patients harbor a homozygous variant in ASPH (NM_004318.3 c.2204 G>A (ep.Arg735Gln)). The substitution of tryptophan for arginine at this residue has been reported previously in Traboulsi syndrome. The ocular findings in Family 1 included both microspherophakia and ectopia lentis. The affected patient in Family 2 (Patient 2-I) has a dilated aortic root (aortic sinus 27.4 mm, Boston z-score of 4.1 and sinotubular junction at 20.9 mm, Boston z-score 3.2). Patient 2-I has a homozygous nonsense variant c.2181_2183dupATG (p.W728X) in ASPH discovered on whole exome sequencing. Comprehensive testing of FBN1 was negative in both families. The phenotype of this extremely rare disorder is continues to evolve and affected patients require a thorough cardiac assessment.

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P05.06B
Metagenomic search for the protozoa in atherosclerotic plaques

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Background: Several reports have shown the relevance of infection in development of atherosclerosis. However, the bacterial DNA of atherosclerotic plaques mainly was investigated by metagenomic approaches. Objective: To search for the protozoa by using genome-wide sequencing data of human atherosclerotic plaques (AP).

Material and Methods: We used the results of whole genome sequencing of DNA isolated from 12 atherosclerotic plaques (SRA149235) and 2 blood samples from 1000 Genomes Project (HG00096, HG00099). Sequence reads were aligned to the human reference genome (hg19) using Bowtie2. The taxonomic classification of the reads was performed with Kraken2 using a curated microbial genome database containing archaea, bacteria, fungi, protozoa, viruses reference genome sequence. Results and discussion: Pre-alignment on the human genome, even with "very-sensitive" option of Bowtie2, cannot completely clean up human gene sequences. Kraken2 determined 13% to 92% of the human gene sequences in unmatched reads of different AP samples. We found sequences of Toxoplasma gondii genome in 8 samples (about 1678 reads per sample). These sequences were shown to be mapped to contigs (NW_017384310.1, NW_017384809.1). These contigs were also found in samples from 1000 Genomes Project.

Conclusions: There were no protozoa in genome-wide sequencing data of human atherosclerotic plaques. The reads that mapped to the Toxoplasma genome are false positive. Some microbial reference genomes probably to contain human DNA sequences not presented in the human reference genome assembly.

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P05.07C
Gain of function mutation in the cardiac Kv4.2 potassium channel underlies paroxysmal atrial fibrillation

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Introduction: Three generations of a kindred presented with autosomal dominant early-onset paroxysmal atrial fibrillation (pAF), with recurrent nocturnal self-terminating palpitations.

Methods: Whole exome sequencing, linkage analysis, electrophysiological assays in *Xenopus* oocytes.

Results: Through genetic studies we identified a disease-causing p.S447R mutation in *KCND2*, encoding the pore-forming (α) subunit of the Kv4.2 cardiac potassium channel. Kv4.2, with Kv4.3, contributes to the cardiac fast transient outward K+ current, I_{to}. I_{to} underlies the early repolarization phase in the cardiac action potential, setting the initial potential of the plateau phase and governing its duration and amplitude. In *Xenopus* oocytes, the p.S447R mutation increased inactivation time constant of the channel and affected its regulation: the mutation resides in a protein-kinase C (PKC) phosphorylation site, which normally attenuates Kv4.2 membrane expression. Mutant Kv4.2 exhibited impaired response to PKC, resulting in augmented Kv4.2 membrane expression and enhanced potassium currents. Moreover, in a hybrid channel composed of Kv4.3 and Kv4.2, simulating the mature endogenous hetero-tetrameric channel underlying I_{to}, the Kv4.2 mutation exerted gain-of-function effect on Kv4.3. Thus, the mutation exerts gain-of-function effect on both Kv4.2 homo-tetramers and Kv4.2-Kv4.3 hetero-tetramers.

Conclusions: Gain-of-function mutation in Kv4.2 causes nocturnal pAF. Interestingly, Kv4.2 expression was previously shown to demonstrate circadian variation, with peak expression at daytime in murine hearts (human nighttime), with possible relevance to the nocturnal onset of paroxysmal AF symptoms in our patients. The atrial-specific phenotype suggests that targeting Kv4.2 might be effective in the treatment of nocturnal paroxysmal AF, avoiding adverse ventricular effects.

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P05.08D
Novel *ABCC9* missense mutation in a Chinese infant with Cantu syndrome without skeletal manifestations

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Background: Cantu syndrome (CS) is a congenital rare disorder characterized by congenital hypertrichosis, neonatal macrosomia, a distinct osteochondrodysplasia, and cardiomegaly. It is a dominant condition caused by heterozygous variants in *ABCC9* or *KCNJ8*. Here, we report a baby boy patient who was referred to Cantu syndrome without skeletal manifestations.

Methods: Blood samples and clinical data were collected after informed consent. Trio whole exome sequencing was performed in this patient and his parents. Public population SNP databases (dbSNP147, gnomAD, ExAC and 1000 Genomes project) and *in silico* predictive algorithms were used to identify the pathogenic variants.

Results: We detected a novel *de novo* heterozygous missense, likely pathogenic variant (c.3203T>C) in *ABCC9* gene. This mutation was located in transmembrane cytoplasmic domain 2 (TMD2) which may be gain-of-function and increase K_{ATP} channel activity.

Conclusion: This is the first report of a Chinese baby patient with pathogenic mutation in *ABCC9*. Our description of the patient's phenotype would be a part of a spectrum of features associated with Cantu syndrome. The unreported mutation extends the genetic spectra of *ABCC9* and emphasizes the usefulness of WES for genetic diagnosis in clinical context. **Keywords:** Cantu syndrome, *ABCC9* gene, whole exome sequencing, cardiovascular abnormalities.

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P05.09A

RASA1 mosaic mutations in patients with capillary malformation - arteriovenous malformation

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Background: Capillary malformation-arteriovenous malformation is an autosomal dominant disorder, characterised by capillary malformations and increased risk of fast-flow vascular malformations, caused by loss-of-function mutations in the *RASA1* or *EPHB4* genes. Around 25% of the patients do not seem to carry germline mutation in either one of these two genes. While some of those 25% of patients may have mutations in as-yet-unidentified genes, mutations in *RASA1* or *EPHB4* that escape detection by less sensitive techniques, such as post-zygotic mosaic mutations, are also possible explanations.

Methods: DNA was extracted from peripheral blood lymphocytes, saliva or vascular malformation tissues from 4 patients. *RASA1* and *EPHB4* coding regions and exon/intron boundaries were analysed by targeted custom gene panel sequencing. A second panel and/or Sanger sequencing were used to confirm the mutations identified.

Results: Four distinct mosaic *RASA1* mutations, with an allele frequency ranging from 3% to 25%, were identified in the 4 index patients with classical capillary malformation - arteriovenous malformation phenotype. Three mutations were known, one was novel. In one patient, a somatic second-hit was also identified. One index case had three affected children, illustrating germline mosaicism.

Conclusion: This study shows that *RASA1* mosaic mutations can cause capillary malformation - arteriovenous malformation. Thus, highly sensitive sequencing techniques should be considered as diagnostic tools, especially for patients with no family history. Even low-level mosaicism can cause the classical phenotype and increased risk for offspring. In addition, our study further supports the second-hit pathophysiological mechanism to explain the multifocality of vascular lesions in this disorder.

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P05.10B

Unique cardiogenetic clinic- our experience from 300 patients from Israeli diverse ethnic population

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Introduction: Genetic heart diseases are a major public health problem worldwide. Cardio-genetics is emerging as a main discipline for deciphering multiple cardiac pathologies, with immediate clinical effects on diagnosis, treatment, prevention and prognosis for patients and their family members.

Methods: We have established the first designated cardio-genetics clinic in Israel as part of a tertiary hospital two years ago. We retrospectively reviewed all our patient's files from the last 2 years.

Results: Three hundred patients from 200 families visited our clinic and they represent variant population from all over Israel both from Jewish and non Jewish origins. The patients were referred from both pediatric and adults cardiologists, cardiovascular surgeons and primary care physicians.

Most visits (90%) in our clinic were as outpatients and only 10% were acutely hospitalized.

The patients were referred due to: congenital heart diseases in 10%, hypertrophic cardiomyopathy in 52%, dilated cardiomyopathy in 14%, arrhythmogenic right ventricle dysplasia in 8%, left ventricular non- compaction in 4%, channelopathies in 10% and 2% due to aborted sudden death.

The patients underwent a genetic investigation in different methods according to the clinical presentation: Sanger sequencing for known familial/founder mutation, single gene sequencing, NGS based gene panel sequencing, Whole Exome Sequencing and chromosomal microarray.

We have identified the underlying genetic cause in approximately 40% of the families - the percentage varies between the different cardiac pathologies.

Summary: Cardio-genetics clinic is a useful tool both for the patient, families and the primary care physician in various Genetic heart disease.

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P05.11C
Molecular diagnostic testing of inherited cardiomyopathies by targeted Next-Generation Sequencing in a public hospital

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Introduction: Inherited cardiomyopathies (CMs) are a major cause of heart disease. They can be classified in hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), restrictive cardiomyopathy (RCM) and left ventricular non-compaction (LVNC). Due to their clinical and genetic heterogeneity, targeted-gene Next-Generation-Sequencing (NGS) can be a helpful tool in their diagnosis and to early detect them in pre-symptomatic carriers.

Materials and Methods: 39 DNA samples of patients affected with different CMs were analyzed by targeted-NGS using Cardio-GeneSGKit® (n=238 genes) or Cardio-GeneSGKit® MCP (n=90 genes). Results were filtered and analyzed with GeneSystems® platform and informed to patients. Co-segregation analysis and/or carrier testing were performed when possible.

Results: 7 pathogenic/probably pathogenic variants were detected in 5 HCM patients and 2 DCM patients. In HCM patients, mutations were found in *MYBPC3* (p.Arg495Gln, p.Gly532Alafs*23 and p.Arg597Gln), *CAV3* (p.Thr78Met), *TNNC1* (p.Ala8Val) and *TNNI3* (p.Arg145Gln). In DCM patients, mutations were detected in *DSP* (p.Leu1773-Tyrfs*8) and *TTN* (p.Pro28946Leufs*6). Also, 17 variants of unknown significance (VOUS) were detected in 14 patients affected with HCM (n=10), DCM (n=3) and ARVC (n=1). These VOUS were mainly detected in *MYBPC3* (n=7) but also in other genes as *DSC2* (n=1), *FHL1* (n=1), *FLNC* (n=1), *LDB3* (n=1), *LMNA* (n=1), *MYH7* (n=2), *PKP4* (n=1), *SCN5A* (n=1) and *TNNT2* (n=2).

Conclusions: 19 of the 39 analyzed patients (48.7%) presented a clinically significant variant in one or two genes associated with CMs. Detection of pathogenic/probably pathogenic variants in probands can help to improve the diagnostic, prognostic and treatment in them and in pre-symptomatic carriers.

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P05.12D
Something old, something new: two syndromes associated with pediatric-onset cardiomyopathy

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Introduction: Hypertrophic and dilated cardiomyopathies (HCM/DCM) are inherited heart muscle disorders. They are isolated in the majority of patients, but are often accompanied by extracardiac features in children. Distinguishing children with one syndromic disorder from those with two inherited conditions is important because this may affect management options, cardiac surveillance in relatives and reproductive decision-making.

Methods and Results: We present two patients with intellectual disability (ID) and cardiomyopathy. Patient 1 presented with paroxysmal ventricular tachycardia at age 3 months. At age 5 she showed severe HCM, prolonged QTc, severe ID, epilepsy and cerebral vision impairment. Exome sequencing (ES) revealed a damaging c.247C>T, p.(Arg83Cys) variant in *NAA10*, which encodes a ribosomal protein involved in N-terminal acetylation. Several X-linked *NAA10* variants have been associated with genetic disorders, but cardiomyopathy had not described until recently. Patient 2 was diagnosed with severe DCM at age 4 months, mild ID, microcephaly (-6.2SDS), failure to thrive and clinodactyly. ES revealed compound heterozygous variants in *CEP135*. Biallelic variants in *CEP135* have also been reported in two families with microcephaly. Interestingly, most of Patient 2's clinical features overlap with those of five previously reported unrelated patients with autosomal recessive inherited microcephaly-cardiomyopathy syndrome with unknown genetic etiology. Genematcher identified another patient with neonatal cardiomyopathy, failure to thrive, microcephaly and biallelic variants in *CEP135*. *CEP135* may thus have a role in cardiomyopathy.

Conclusions: These two cases illustrate that ES is a valuable diagnostic tool for pediatric-onset cardiomyopathy that can identify novel features in well-known syndromes or novel genes associated with pediatric cardiomyopathy.

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P05.13A
Cardiomyopathy: genomic-diagnostic approach

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Cardiomyopathy is a disease of the heart muscle associated with a disorder of its function. This is a heterogeneous group of diseases with various clinical signs that can ultimately lead to heart failure. A significant proportion of cardiomyopathies is genetically determined. In this group can be found congenital heart defects, muscular dystrophy and congenital myopathy, hereditary disorders of metabolism and genetic syndromes, (rasopathies). The use of the whole exome sequencing is currently one of the most effective tools for elucidating the genotype of individual patients. We have successfully used this method for studying of genetic architecture of dilated cardiomyopathy cohort where in about 80% of the 460 examined patients we have found probably causal variant. Most frequently affected genes were *TTN* (18%), *FLNC* (4%), *MYBPC3* (4%), *MYH7* (4%), *DSP* (4%), *RBM20* (3%), *TNNT2*(3%), *DES* (2%), *LMNA* (1%). We have also found CNV variations in *DMD*, *LAMP2*, *FLNC, LMNA* and *MYH7* genes, which were predicted to cause major structural and functional abnormalities of the affected genes. We are also focusing on paediatric forms of cardiomyopathy which are more complex and the clarification is below 50%. Successful molecular biology diagnostic helps to identify the risk of occurrence of the disease in the family and to provide prenatal diagnosis. In selected cases, the phenotype can also be studied in cell models, which contribute to the understanding of the molecular mechanism of the disease and allow a more accurate interpretation of extreme clinical or laboratory findings. LM201509 NCLG, AZV 15-27682A, AZV 15-28208A, SVV-260367

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P05.14B
11 years of the Queensland Cardiac Genetics Service

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Introduction: A dedicated conjoint cardiac genetics clinic (CGC) was established through Genetic Health Queensland and the Cardiology Department at the Royal Brisbane and Women's Hospital in 2007. The aim of this study was to characterize the patient cohort seen and assess the uptake of genetic testing and diagnostic rates achieved.

Methods: We explored the local databases and patient charts to characterise the age, sex, referral phenotype and incidence of genetic testing for all patients who had attended the CGC. The outcomes of genetic testing, and number

of at risk relatives screened were also calculated. Unsolved cases who would benefit from further testing with Whole Genome Sequencing were identified.

Preliminary Results: Over 1400 individuals were seen for the first time during the 11-year period and over 900 have been characterized to date. 488 were probands, the majority (265) of whom were referred for a cardiomyopathy. 141 were referred for an arrhythmia syndrome, and 72 had had a cardiac arrest or were the relative of an individual with a sudden cardiac death. 385 probands were tested, with a genetic diagnosis being achieved in 164 (43%). The highest diagnostic rate was achieved in patients with cardiomyopathies (47%). Predictive testing was performed in a further 415 patients.

Conclusions: We have reviewed the characteristics of a large cohort of cardiac genetics patients and compared our diagnostic rates to those in the literature. We have identified unsolved families who will benefit from further testing with contemporary technologies.

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P05.15C
Structural variants in Alpha-actinin 2 are associated with cardiomyopathy and hypertrophy in human cardiac tissue and iPSC-derived cardiomyocytes

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Background: In striated muscle, alpha-actinin 2 is a critical cytoskeletal protein that anchors actin filaments within the sarcomere. Rare mutations in *ACTN2* have been associated with cardiac abnormalities, including arrhythmias and cardiomyopathy. However, the mechanisms behind how dysfunctional ACTN2 causes cardiac malfunction is not known. The aim of the present study was to investigate the effects of two novel *ACTN2* variants on human cardiac tissue and patient-specific iPSC-derived cardiomyocytes.

Methods and Results: We identified patients in the Stanford Center for Inherited Cardiovascular Disease database with novel *ACTN2* variants using a custom mutation pipeline optimized for rare variant discovery. We identified one patient homozygous for a stop-gain mutation (p. Q860X) in *ACTN2* and a family with an exon 8-10 deletion. In heart transplant tissue of the homozygous patient, we observed mild hypertrophy and interstitial fibrosis. There was no evidence of variation in ACTN2 protein expression, indicating absence of nonsense mediated decay. We used siRNA to knock down ACTN2 in neonatal rat ventricular cardiomyocytes and a human myoblast cell line and observed dramatic changes in cell size and morphology. Patient-derived iPSC-cardiomyocytes were hypertrophic, displayed sarcomeric structural disarray and had slower contractile velocity compared to control iPSC-cardiomyocytes. Using Co-Immunoprecipitation for ACTN2, followed by mass-spectrometry, we identified missing protein-protein interactions in the patient with the truncated ACTN2.

Conclusions: Here, we provide evidence that two structural genetic variants in *ACTN2* are associated to contractile dysfunction and lead to cardiac abnormalities, including hypertrophy and arrhythmia.

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P05.16D
Genetic cardiomyopathies revisited: reevaluating current gene panel testing

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Introduction: There is an ongoing debate on the genes that should be examined during genetic evaluation of idiopathic cardiomyopathy patients. Although several genes have sufficient evidence to be cardiomyopathy-related, for other genes this remains unclear. These genes may however cause a significant burden on variant evaluation. In order to make an evidence-based estimation of the value of genes examined, we assessed results of cardiomyopathy gene panels after reevaluation and reclassification of variants according to current criteria.

Materials and Methods: All results of a 64-gene NGS-cardiomyopathy panel performed between 2014 and 2018 in the UMC Utrecht, the Netherlands were retrieved. The pathogenicity of all gene variants was reevaluated according to current criteria by an experienced clinical laboratory specialist in cardiogenetics. Benign or likely benign variants were excluded from analysis. Summary results were determined for all individual genes.

Results: 1264 cardiomyopathy panels performed showed 848 variants (657 unique) in 638 patients (overall yield 50.5%). Of the 848 variants, 124 variants were reclassified (15%), of which 117 were downgraded (94%). Reclassification was mostly due to a high frequency in control

populations or based on in-house clinical data (e.g. non-segregation). 28 genes were determined to be of limited value in regular pan-cardiomyopathy gene panel testing because of the absence of relevant variants, published literature (including ClinGen gene adjudication).

Conclusions: Cardiomyopathy NGS-gene panels performed on 1264 patients, identified 28 genes of limited value in regular genetic testing for cardiomyopathy. In addition, state-of-art reevaluation of variants resulted in a notable downgrading of previously identified variants.

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P05.17A
Circadian clock genes and myocardial infarction in patients with type 2 diabetes mellitus

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Disruption of circadian clock may trigger the onset of diabetes mellitus and myocardial infarction. Type 2 diabetes mellitus (T2DM) is well-known risk factors for cardiovascular diseases and myocardial infarction. Several physiological factors can stimulate the emergence of T2DM and myocardial infarction, and some of these factors are known to oscillate with circadian rhythms. This study aimed to explore a possible association of the genetic variability in the circadian clock genes *ARNTL*, *CLOCK*, and *PER2* with myocardial infarction in patients with type 2 diabetes mellitus as an additional risk factor for myocardial infarction. The study group consisted of 231 patients with myocardial infarction and T2DM and a control group of 426 T2DM patients. Altogether, 8 SNPs were tested, three in *ARNTL* gene (rs3789327, rs4757144 and rs12363415), three in *CLOCK* (rs11932595, rs6811520 and rs13124436), and two in *PER2* (rs35333999 and rs934945). The significance of association for individual SNP was calculated to compare the allelic frequency and genotype distribution in patients and control participants using the Chi-Square test. After using Bonferroni correction significant difference in the distribution of *ARNTL* rs12363415 polymorphism genotypes were found in patients with myocardial infarction and T2DM in comparison to controls, with a p-value of $1.42x10^{-4}$ and odds ratio equal to 7.37 (95% CI: 4.15 to 13.08). Other SNPs in *ARNTL*, *CLOCK*, and *PER2* genes were not significant additional risk factors for myocardial infarction in T2DM patients. We provide evidence that genetic variation in ARNTL gene might be an additional risk factor for myocardial infarction in T2DM patients.

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P05.18B
Clinical Utility of Next Generation Sequencing (NGS) Panel Testing in Patients with a Clinical Suspicion of Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

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Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a rare, but potentially fatal channelopathy. Genetic testing may be used to confirm a diagnosis in unclear cases and therefore, is increasingly being performed in a heterogeneous patient population. This is a retrospective review of 134 patients with clinical suspicion of CPVT referred for genetic testing at Blueprint Genetics over a 5-year period (2013-2018). Genetic test results were compiled. A sub-analysis of diagnostic *RYR2* variants (location, segregation) was performed. A pathogenic (P) or likely pathogenic (LP) variant was identified in 27 patients (20.1%). Twenty patients (14.9%) had a diagnostic finding in a CPVT-associated gene: 62.9% in *RYR2*, 7.4 % in *CALM1*, and 3.7% in *CASQ2* (biallelic). Four patients (14.8%) had a P or LP variant in *KCNQ1*, *KCNJ2* or *SCN5A* and three (11.1%) had a P or LP variant in a cardiomyopathy-associated gene (*DSG2, DSP* or *PLN*). All P/LP *RYR2* variants were missense, except a deletion encompassing exon 3. Parental testing was performed in 11/17 cases where P/LP *RYR2* variants were found; 8 (72.7%) variants were *de novo*. Enrichment of P/LP *RYR2* variants in the four described hotspots (OR 50, 95% CI 29-85, P<0.0001) was observed. In conclusion, 37% of patients with a diagnostic test result had a clinically significant variant in a gene other than *RYR2*. Interestingly, 26% of these patients had genetic diagnosis of a cardiomyopathy or a channelopathy other than CPVT. This study supports the

utilization of broad NGS panels for patients with a clinical suspicion of CPVT.

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P05.19C
Mutations in genes involved in MAPK pathway cause lymphatic anomalies

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Complex lymphatic anomalies include a variety of diagnoses: lymphangiectasia, central conducting lymphatic anomaly (CCLA), generalized lymphatic anomaly, kaposiform lymphangiomatosis, and Gorham Stout disease. The overlapping of diagnostic criteria for these disorders suggests that a common pathway rather than a common gene is responsible for the various clinical syndromes. Upon sequencing 45 such patients, we identified seven pathogenic variants in five genes encoding components of RAS/MAPK signaling (*BRAF*, *KRAS*, *SOS1*, *RASA1*, and *PTPN11*) in seven unrelated patients. Additionally, we identified a recurrent somatic missense mutation in a candidate gene, *ARAF* (c.640T>C:p.S214P), as the basis for CCLA in two unrelated patients. *ARAF* encodes for serine/threonine-protein kinase A-Raf. Little is known about ARAF apart from the enzyme being involved in MAPK pathway with no previous involvement in lymphatic disease report. Primary endothelial cell studies showed the mutation altered actin skeleton and VE-cadherin organization, which were fully reversed by inhibition of MEK signaling. Functional relevance of the mutation was also validated by recreating a lymphatic phenotype in a zebrafish model, with rescue of the anomalous phenotype using a MEK inhibitor. Subsequent therapy of the lead proband with a MEK inhibitor led to dramatic clinical improvement, with remodeling of the patient's lymphatic system with resolution of the lymphatic edema, marked improvement in his pulmonary function tests, cessation of supplemental oxygen requirements, and near normalization of daily activities. Taken together, our work demonstrates the realization of precision medicine approach enabled through a novel genetic discovery in a patient with lymphatic anomaly of a previously unknown etiology.

D. Li: None.

P05.21A
Copy number variants detection by microarray and multiplex ligation-dependent probe amplification in congenital heart diseases

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Congenital heart diseases (CHDs) are the most common birth defects among life births, which could be presented as isolated or syndromic with other congenital malformations. The etiology of CHD largely unknown, genetic and environmental factors contribute to the disease. Recurrent

copy number variants (CNVs) have been reported in the pathogenesis of CHD. The aim of this study was to evaluate the clinical utility of multiplex ligation-dependent probe amplification (MLPA) and microarray analyses on isolated and syndromic CHD cases and to explore the relationship between identified CNVs and CHD.

Materials and Methods: Cytoscan 750K array (Affymetrix) and MLPA SALSA P250 and P311 kits (MRC-Holland) were used in the study. The identified CNVs were confirmed by fluorescence in situ hybridization. Results. Eighteen prenatal samples, 16 isolated and 33 syndromic patients with mild to severe CHD phenotype were studied. Prenatal and isolated CHD cases did not show pathogenic CNVs. Clinically significant CNVs were detected in 7/33 (21%) syndromic CHD patients: del 22q11.2 (n=2), 8p23.1 duplication (n=2), deletion 5p (n=1), deletion 6q21-q22 (n=1), unbalanced translocation causing partial deletion of 4q34.3 and duplication of 6q25.1 (n=1).

Conclusion: The identified genomic imbalances contain genes that has been associated with human CHD before. The present study demonstrates that using microarray and MLPA analysis increases the detection rate of causal CNVs in individuals with syndromic CHD. This study was supported by the Ministry of National Economy, Hungary (GINOP-2.3.2-15-2016-00039).

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P05.24D

Adipokine profile of adipocytes isolated from different fat depots of coronary artery disease patients

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Introduction: Adipose tissue is interesting in the context of its role in pathogenesis of cardiovascular diseases. Human adipokinome consists of hundreds of molecules many of which have not yet been well characterized and have variable expression in different adipose depots.

Materials and Methods: We compared *DPP4*, *LCN2*, *NAMPT*, *ITLN1*, *APLN* and *ADIPOQ* mRNA levels in adipocytes isolated from the biopsies of subcutaneous (SCAT), epicardial (EAT) and perivascular (PVAT) adipose tissue obtained from 25 patients with coronary artery disease. Gene expression signature was determined by RT-qPCR with hydrolysis probes.

Results: We found that *DPP4* and *APLN* mRNA expression was significantly ($P<0.05$) increased only in adipocytes isolated from epicardial adipose tissue compared to the subcutaneous fat. The *ITLN1* gene was overexpressed in epicardial adipose tissue compared to both subcutaneous and perivascular tissues and expression of *ADIPOQ* gene was reduced both in EAT and PVAT compared to SCAT. Moreover, *APLN* mRNA expression was positively correlated with total and LDL cholesterol plasma level, and *DPP4* mRNA expression - with VLDL cholesterol concentration. We found no significant changes in *LCN2* and *NAMPT* mRNA level between studied adipose depots.

Conclusions: Adipocytes isolated from different adipose depots are characterized by differential gene expression of adipokines. EAT is of particular interest in the context of its function, molecular and genetic mechanisms of regulation of the cardiovascular system and as a therapeutic target for correction of adipose tissue-induced effects on health. This work was supported by RSF grant No. 17-75-20026.

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P05.26B

Cardiac arrhythmias at baseline predict a clinically relevant genetic yield in idiopathic non familial DCM affecting long term outcome

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Background: Current guidelines recommend genetic analysis only in familial dilated cardiomyopathy (DCM) patients without acquired disease. The genetic yield in so-called acquired and/or non-familial DCM remains largely unknown.

Methods: This study included 689 DCM patients who all underwent genetic evaluation using a 47 cardiomyopathy-associated gene panel and had complete cardiac diagnostic work-up including echocardiography, cardiac MRI, endomyocardial biopsies and holter monitoring. Environmental triggers were predefined as viral, inflammatory, toxic, electrical and systemic auto-immune disease.

Results: At least one etiology (genetic and/or environmental) was found in 530 patients (77%) after complete

diagnostic workup, of which 159 (23%) had a genetic mutation. One in five of the DCM patients (n=90; 20%) had a combination of a genetic and acquired (environmental) trigger. All of the acquired triggers had a genetic yield around 20%. Although familial DCM had a significant higher yield of genetic mutations compared to non-familial DCM (43% versus 16%, p<0.001), pathogenic mutations were still present in 1 of 6. In non-familial DCM patients with arrhythmias at baseline without environmental trigger, the genetic yield was even 39%, comparable to the yield in familial DCM (43%; p=0,17). Also, genetic DCM has a worse prognosis compared to non-genetic DCM (Log-Rank=0.008), irrespective of familial history (Log-Rank=0.29).

Conclusions: One in five DCM patients with an acquired trigger and/or absence of familial history has an underlying genetic mutation. Non-familial DCM patients with arrhythmias and no clear etiology have a comparable genetic yield compared to familial DCM.

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P05.27C

A novel truncating mutation in *DSP* (Desmoplakin) causes autosomal dominant dilated cardiomyopathy with variable penetrance

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Background: Arrhythmogenic Cardiomyopathy (AC) is mainly determined by mutations in desmosomal genes. Approximately 75% of AC patients show bi-ventricular involvement and left-dominant forms are not uncommon. Loss-of-function mutations in Desmoplakin (*DSP*) cause a form of AC often manifesting with severe arrhythmias and sudden cardiac death (SCD). Less is known about *DSP* mutations in dilated cardiomyopathy (DCM) regarding (endo)phenotype, penetrance, and management.

Materials and Methods: A family with DCM and SCD was referred to our cardiogenetics outpatient clinic. Accurate genetic and cardiologic assessments were performed. DNA sequencing was initiated in the proband using NGS (cardiopanel).

Results: A novel variant in *DSP* c.4639dupC was identified, absent in gnomAD, locus-specific databases and predicted as deleterious by dedicated software, affecting cardiac-specific isoform and leading to frameshift p.(Gln1547ProfsTer80). The 60-year-old proband and her three nieces (aged 32-45) were diagnosed with DCM and ICD implanted. Five siblings (aged 25-61) had SCD. Segregation analysis in asymptomatic relatives identified two mutated siblings (31 and 47 years): one presented an episode of acute myocarditis. Despite detailed cardiologic evaluation, they did not meet current diagnostic guidelines for AC/DCM.

Conclusions: Loss-of-function *DSP* mutations cause different phenotypes including AC and DCM. A diagnostic grey zone between left-dominant AC and arrhythmogenic DCM exists, indicating the need for updating current classification. Moreover, genotype-based definition is warranted to develop personalized management in patients/asymptomatic-mutated subjects. Intrafamilial phenotypic variability and lack of penetrance demand modifier variants in additional, yet unknown, genes or epigenetic factors.

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P05.28D

Double heterozygosity in a group of patients with rare cardiovascular diseases

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Introduction: Rare cardiovascular diseases (CVD) are heterogeneous and this complicates their diagnosis. The more complex forms of rare CVD tend to result from the integrated effect of multiple genetic variants that create a background of susceptibility requiring sometimes additional factors for the disease to manifest. Whole exome, whole genome and target sequencing offer new possibilities to discover the complex etiology of such diseases. The aim of this study was to identify genetic variants associated with the rare CVD using next generation sequencing (NGS).

Materials and Methods: NGS with TruSight panels (Illumina) was used to analyze DNA samples from 24 patients with rare CVD: Long QT Syndrome, Tetralogy of Fallot, Familial Hypercholesterolemia, suspected Marfan Syndrome, Pathology of great vessels, Cardiomyopathies and other.

Results: In 14 (58%) of the 24 patients, at least one pathogenic or likely pathogenic variant was found. Of those, in 8 patients we detected only one variant, in 3 patients there was a combination of two or more only pathogenic/likely pathogenic variants, and in 3 patients the combination was of pathogenic/likely pathogenic and VUS. Of the remaining 10 patients, in 5 (21%) patients, only VUS were detected (3 with two VUS and 2 with one VUS) and in 5 (21%) patients, we did not detect significant mutations. Overall, in 9 (47%) of the patients with detected variants, more than one variant was found indicating multiple heterozygosity. In conclusion, NGS has a potential to detect multiple heterozygosity in complex rare cardiovascular diseases.

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P05.29A

The causal relationship between dyslipidemia and cardiovascular disease using Mendelian randomization

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Background: Cardiovascular disease (CVD) is ranked as the second leading causes of death in Taiwan. Dyslipidemia is one of the risk factors for CVD. The *LPL* and *APOE* genes were found to be involved in lipid metabolism. Many studies uncovered the association between dyslipidemia and CVD, however, conventional epidemiological studies may suffer from un-measured confounders and reverse causality. We aimed to elucidate the causal relationship between dyslipidemia and CVD using Mendelian randomization design.

Methods: A total of 1951 Chinese adults were recruited from the Matsu community-based integrated health screening project during 2015-2017. Two SNPs (*LPL* rs328 and *APOE* rs157580) were selected as the instrumental variables. Both logistic regression and two-stage least-square instrumental variable (IV) regression were applied to estimate the relationship between the lipid profiles and CVD.

Results: We found rs328 was significantly associated with decreased triglyceride levels (β=-9.105, p=0.012) and increased HDL-C levels (β=1.317, p=0.037), but not with LDL-C levels. The rs157580 was marginally associated with increased LDL-C levels. We further found a significantly protective effect of LDL-C levels on cardiovascular disease (OR=0.987, 95% CI=0.980-0.994), but not with triglycerides, HDL-C and CVD. The IV analysis revealed an increasing risk of LDL-C levels on cardiovascular disease by using rs157580 and allele score as the IVs (rs157580: OR=1.028, 95% CI=1.018-1.038; allele score: OR=1.028, 95% CI=1.019-1.038). Triglyceride and HDL-C were also associated with cardiovascular disease.

Conclusion: We identified the causal relationships between dyslipidemia and cardiovascular disease by using *APOE* rs157580 and allele score as the instrumental variables. Grant No: MOST 106-2314-B-010-020-MY3

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P05.30B

Common atrium, atrioventricular canal defect/postaxial polydactyly: a mild clinical subtype of Ellis-van Creveld syndrome caused by compound heterozygosity for loss of function and hypomorphic *EVC* mutations

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Clinical expression of Ellis van Creveld syndrome (EvCS) is variable with a classic form associated with homozygous/compound heterozygous mutations in *EVC* and *EVC2* genes, and the milder Weyers acrodental dysostosis linked to specific heterozygous mutations in the last coding exon of *EVC2*. Additional transitional phenotypes have been described, including patients with prevalently cardiac and limb involvement, presenting with atrioventricular canal defect with common atrium (AVCD-CA) and postaxial polydactyly. We report on the results of molecular analysis of a family with vertical transmission in father and daughter of AVCD-CA and postaxial polydactyly. The father was compound heterozygous for the known recessive p.Arg622* nonsense variant and the novel p.Arg663Pro missense change in the *EVC* gene. His affected daughter was compound heterozygous for the same nonsense variant and the novel splice-site c.1316-7A>G variant. Two additional unaffected sisters were also examined. One was heterozygous carrier of the p.Arg622* variant, while the other was compound heterozygous for both the p.Arg663Pro change and the c.1316-7A>G variant. mRNA sequencing in the affected daughter's fibroblast showed that c.1316-7A>G resulted in the in-frame insertion of 6 nucleotides between *EVC* exon 9 and 10, and that the expression of the p.Arg622* corresponding allele was greatly decreased. Consistently, western blot analysis showed a residual amount of EVC protein in the father's and daughter's fibroblasts. Present results suggest that p.Arg663Pro and c.1316-7A>G are hypomorphic *EVC* alleles that act as genetic modifiers together with a loss of function mutation to cause a milder clinical subtype of EvCS characterized by AVCD-CA and postaxial polydactyly.

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P05.31C

Heritability and family-based GWAS analyses of the circulating ceramide, endocannabinoid, and N-acyl ethanolamide lipidome

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Introduction: Lipids of the endocannabinoid (EC), N-acyl ethanolamine (NAEA), and ceramide (CER) classes are potential novel biomarkers of coronary artery disease and type-2 diabetes. Major-gene effects have been discovered for certain lipid species, notably lipoprotein(a). We sought to establish the heritability of EC, NAEA, and CER species, and identify DNA variants influencing their concentrations in plasma.

Materials and Methods: We undertook heritability (QTDT, GCTA) and GWAS analyses (FaST-LMM) of 11 ECs and NAEAs, and 37 CERs in 1,016 plasma samples from 196 British Caucasian families ascertained through a hypertensive proband, using targeted mass spectrometry and Illumina 660W-Quad genotyping.

Results: NAEAs are more heritable than well-studied EC anandamide (AEA) (h^2AEA = 32-35%; $P<5.80\times10^{-11}$). 24-46% of the variation in potential biomarker CER is due to genetic factors ($P<1.00\times10^{-7}$). GWAS identified associations with eQTLs of proteins in their metabolism (e.g. FAAH; P_{NAEA}DHEA $<6.33\times10^{-12}$, SPTLC3; P_{CER} N(24)S(18) $<8.99\times10^{-19}$) and novel loci implicated in cancer risk and non-alcoholic fatty liver disease (e.g. FBXO28; P_{CER}N(24)S(19)ratio $<1.95\times10^{-8}$, SULT1C4; P_{CER}N(24)S(19) $<8.99\times10^{-19}$). Two-sample Mendelian randomisation suggests that a variant in FAAH (rs324420) influencing the level of plasma NAEAs ($beta_{NAEA}$DHEA = 0.39; $P<6.33\times10^{-12}$) is causally associated with both obesity and drug addiction.

Conclusions: We demonstrate for the first time estimates of heritability for this extended array of bioactive lipids, identify GWAS-significant SNPs associating with their levels in circulation, and implicate the lipid species studied here in cardiovascular disease, cancer, and drug addition.

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P05.32D

A new zebrafish model for *in vivo* optical mapping of cardiac action potentials

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Genetically encoded voltage indicators (GEVI) allow optical mapping of voltage changes and offer an alternative method for measuring alterations in cell membrane potentials. Initially driven by the neuroscience field, the development of GEVIs has now found applications in the cardiovascular sciences as well. Thanks to these GEVIs, it has become possible to image cardiac voltage dynamics at the cellular level *in vivo* in translucent organisms such as zebrafish. Unfortunately, up to now, the fluorescent signal of the cardiac voltage sensors used in zebrafish has been very dim. For this reason, we developed a new stable transgenic zebrafish model, expressing Ace2N-mNeon, a next generation voltage sensor with brighter fluorescence, under the control of the myocardial specific promoter *myl7*. Using Selective Plane lllumination Microscopy (SPIM), the conduction of individual action potentials in the heart of 3 dpf zebrafish larvae can be visualized *in vivo*, allowing the measurements of action potential duration and conduction speed. Next, we will apply this new transgenic zebrafish model to evaluate the pathogenicity of genetic variants of unknown significance identified in arrhythmia genes of patients with inherited primary electrical diseases.

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P05.33A
Diagnostic Yield of Genetic Testing in an Unselected Cohort of 1,376 HCM Patients

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Introduction: Genetic testing in Hypertrophic Cardiomyopathy (HCM) is recommended by published guidelines. Genetic testing by NGS panels offers practical differential diagnostic solution.

Objective: Diagnostic yield in a heterogeneous cohort of patients with a suspicion of HCM

Methods: A retrospective review of patients with a suspected clinical diagnosis of HCM referred for genetic testing at Blueprint Genetics between 2013 and 2018 was undertaken. Variants classified as pathogenic (P) or likely pathogenic (LP) at the time of reporting were considered diagnostic.

Results: Diagnostic yield was 26.2% (361/1,376). In total, 373 P/LP variants were identified including 363 variants diagnostic for HCM whereas only 10 were diagnostic for another type of cardiomyopathy based on clinical and genetic interpretation (*DES, DSP, LMNA, TTN*). Approximately 86% (n=320) of diagnostic variants (23.3% of all tests) involved genes encoding the sarcomere. Seventeen P or LP variants (4.6% of variants and 1.2% of all tests) were in RASopathy genes and thirteen P or LP variants (3.5% of 0.9% of all tests) were in metabolic/infiltrative disease genes. In addition, three patients with non-diagnostic findings for cardiomyopathy had P or LP variants in genes linked to a channelopathy *(RYR2, SCN5A)* or neurofibromatosis (*NF1*).

Conclusions: The diagnostic yield of genetic testing in a heterogeneous cohort of patients with a suspected diagnosis of HCM analyzed is lower than what has been reported in well characterized patient cohorts. Importantly, 8% of all diagnostic findings were in metabolic and RASopathy genes which have significant systemic medical management implications.

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P05.34B
Psycho-social impact of predictive genetic testing in hereditary heart diseases (PREDICT Study)

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Introduction: Hereditary heart diseases are most often characterized by autosomal dominant inheritance and delayed cardiac expression. Predictive genetic testing is offered to asymptomatic relatives to allow targeted medical care with early therapeutics in order to reduce the risk of complications. The aim of this study was to evaluate the psychological and socio-professional impact of predictive genetic testing in hereditary heart diseases.

Patients and Methods: This multicentric French study involved 20 expert centers in hereditary heart diseases. We included 517 adult relatives (42.3±16.7 years, 60.6% females) who performed predictive genetic testing (prospective study: N=264, retrospective study: N=253). The opinion and experience were collected via auto-questionnaires, at various moments in the prospective study, with different items and validated scales (STAI and IES).

Results: In the prospective study, the main motivations for performing the test were: "to remove doubt" (65.3%), "for children" (64.0%), "to benefit from medical supervision" (34.9%). A mutation was present in 39.4% of relatives. No regret was expressed after testing (only 2.3% regrets). The result did not lead to a socio-professional change or family relationship change in 60.7%. Among those who had a change, it was perceived as unfavorable for only 3%. The level of anxiety (STAI scale) increases before the test result and decreased to return to baseline. Subjects with depression history were more likely to develop anxiety at long term (p=0.004).

Conclusions: Our results show no or marginal adverse psychological and socio-professional impact of genetic testing when performed by a team expert in predictive testing.

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P05.35C

Development of a fast and cost effective genetic diagnostic method for familial hypercholesterolemia in Sweden

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Familial hypercholesterolemia (FH) is an autosomal dominant disease causing elevated levels of low-density lipoprotein cholesterol, carrying a high risk of premature coronary heart disease such as myocardial infarction. The prevalence of FH is approximately 1/250. There is an obvious need for improving early detection and treatment of FH, which is currently an underdiagnosed condition.

We aimed to characterize the disease-causing mutation spectrum in Sweden, and to develop a cost-effective diagnostics. This was done through iterative improvement of the analytical strategy, consisting of (i) targeted mutation analysis using a panel based on Agena mass spectrometry-based genotyping; (ii) sequencing of samples failing to show mutations; and (iii) redesign of the panel to include new, recurrently found mutations.

To date, we have included 1,143 patients from 14 hospitals across Sweden in a nationwide collaboration using a panel of 113 pathogenic/likely pathogenic mutations in the *LDLR*, *PCSK9* and *APOB* genes.

Dutch Lipid Clinical Network (DLCN) score was available for 482 patients in whom mutations were detected in 27%. If restricted to patients with probable or definite FH (score ≥6; n=309) pathogenic mutations were detected in 35%. The two most prevalent mutations were NM_000384.2(APOB):c.10580G>A(p.Arg3527Gln) and NM_000527.4(LDLR):c.259T>G(p.Trp87Gly). In total, 38 different mutations were detected by the panel. 142 panel negative samples were sequenced with the SEQPPRO-LIPO method, yielding 15 additional mutations in 16 patients.

In conclusion, our FH-panel detected mutations in approximately one fourth of Swedish patients with suspected FH. The number was increased if analyses were restricted to patients with high DLCN score.

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P05.36D
A novel candidate gene of familial hypertrophic cardiomyopathy with therapeutic potential

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TNNI3K encodes cardiac troponin I-interacting kinase (TNNI3K), forming homo-dimers or homo-oligomers and involving in the cardiac contractility regulation. Loss-of-function mutations in *TNNI3K* can lead to dilated cardiomyopathy (MIM616117). No *TNNI3K* mutations have been found to cause hypertrophic cardiomyopathy in (HCM: MIM192600). We performed a 178 gene-panel enriched and next-generation sequencing in 8 Thai families with HCM. 8 pathogenic (P)/likely pathogenic (LP) variants were found in 7 families (*MHY7(4)*, *TPM1(2),TNNI2(1), TTR*(1)). One family harbored two variants in *MYH7 and TPM1*. The remaining family without identified P/LP variants was sent for whole-exome sequencing. We identified a rare spliced *TNNI3K* (c.1178-2A>G) variant in the 41-year-old male with severe eccentric HCM who presented with syncope from ventricular arrhythmia. His asymptomatic 60-year-old father with apical HCM also harbored this variant. The variant was observed once in the ExAC database and once in our in-house Thai-exome database (>2,000 cases). RNA analysis revealed an altered splicing mRNA predicted to create a protein with only N-terminal Ankyrin repeats without the kinase and the C-terminal serine-rich domain. Since the C-terminal serine rich domain is an inhibitory domain, the identified mutation may represent a gain-of-function variant by diminishing inhibitory signal of the kinase domain. A previous study expressing wild-type human TNNI3K in transgenic-mice found that the mice had cardiac hypertrophy. TNNI3K inhibitor which has undergone preclinical testing can serve as a potential therapy for patients with HCM. In summary, we report a family with a probably gain-of-function mutation in *TNNI3K*, suggesting that TNNI3K is a new disease gene for HCM.

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P05.37A
Circulating miRNAs profiling in diabetic patients with ischemic heart disease

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Introduction: Due to a global increase of morbidity and mortality with ischemic heart disease (IHD) in type 2 diabetic patients, there is an urgent need to identify of early biomarkers, which would help to predict an individual risk of development of IHD. MiRNAs are small noncoding RNAs which regulate gene expression and the last few

years appear as a key tool for understanding the pathophysiology of IHD. Here, we postulate that circulating serum-derived miRNA may serve as potential biomarkers for early IHD diagnosis and help to identify diabetic individuals with a predisposition to develop ischemic heart disease.

Materials and Methods: We obtained serum samples from 39 T2DM patients (22 with IHD and 17 free of complications). The levels of 798 miRNAs were analysed using NanoString nCounter Technology Platform. The miRNA regulatory network analysis was performed using the Ingenuity Pathway Analysis software.

Results: Our data showed that 28 miRNAs (including miR-615-3p, miR-1303, miRR-122-5p, miR-217) were significantly upregulated and 1 miRNA (miR-451a) was downregulated in T2DM IHD patients compared to T2DM patients without IHD (adj $p < 0.05$). Based on the above all miRNAs were classified into four interactive signalling networks. Targeted genes by the identified miRNAs were enriched in pathways associated with metabolic and cardiovascular diseases, cardiac dysfunction and cardiovascular system development.

Conclusions: Taken together, our findings suggest that circulating miRNAs might have a crucial role in the development of IHD in diabetic patients and may be used as a potential for early diagnosis.

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P05.38B
Association of polymorphisms in PDE4D, ACE and GP1BA genes with clinical characteristics in patients with ischemic stroke and chronic cerebral ischemia

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Introduction: A large data has been accumulated about the role of genetic factors in the development of cerebrovascular diseases, but the identified associations are often not reproducible in replication studies and meta-analyses. This may be due to the multifactorial nature of this group of diseases, the heterogeneity of pathogenic mechanisms, and the ethnicity of patients. The work was aimed to investigate the association of genetic polymorphisms with patient's characteristics in well-defined clinical groups.

Patients and Methods: The study included 81 patients with chronic cerebral ischemia (without a history of stroke) and 69 patients with ischemic stroke (IS). Genotyping was performed using microarray to determine 21 SNPs in the ACE, SERPINE1, FGB, F5, F7, F12, GP1BA, GPIIIa, MTHFR, CYP11B2, PON1, PON2, NOS2, NOS3, PDE4D, HIFla, LTA, ALOX5AP genes. Stroke subtypes were defined according to TOAST criteria.

Results: In patients with chronic cerebral ischemia, the TT genotype of the PDE4D (rs966221, SNP83C>T) was associated with rapidly progressive arterial hypertension (OR=6.22; 95%CI=1.9-20.8; p=0.0036). In patients with IS the D allele of the ACE gene (rs1799752, I>D) and the DD genotype were associated with cardioembolic subtype of stroke (OR=2.67, 95%CI=1.23-5.8, p=0.02 and OR=7.14, 95%CI=1.7-29.7, p=0.0057). In patients with IS the C allele of the GP1BA gene (rs2243093, -5T>C) and TC genotype were associated with large-artery atherosclerosis subtype of stroke (brachiocephalic artery stenosis >75% and large-artery occlusion) (OR=3.39, 95%CI=1.1-10.2, p=0.03 and OR=4.44, 95% CI=1.3-15.5, p=0.023).

Conclusions: Detailed genetic analysis in the context of clinical features of cerebrovascular disease will allow the identification of significant associations.

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P05.39C
Apolipoprotein E e4 associates to age of ischemic stroke onset but not to stroke outcome

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Introduction: APOE genotype is a candidate predictor for stroke outcomes. We evaluated whether common APOE alleles associate with ischemic stroke outcome, severity and age at onset, within the large multicenter Genetics of Ischaemic Stroke Functional Outcome (GISCOME) study.

Methods: This study comprises 6,165 ischemic stroke cases with genotype data from SNP arrays. Baseline stroke severity was scored according to the NIH Stroke Scale (NIHSS). Functional outcome at 3 months was scored according to the modified Rankin Scale (mRS). We derived common APOE allele status (ε2,ε3,ε4) by combining information from SNPs rs7412 and rs429358. Effects of minor allele count (ε4,ε2) on age at stroke, stroke severity and outcome were estimated separately in comparison to the most common ε3/ε3 genotype.

Results: We found no evidence of a direct effect of ε4 on outcome. There was, however, an inverse association between ε4 allele count and age at stroke (β-1.8, P=0.00017). This association was significant in both sexes. The ε2 allele was independently associated with poor functional outcome (mRS>2) in men (odds ratio[OR]1.47, P=0.008), but not in women (OR 0.87, P=0.44).

Conclusion: This is the largest meta-analysis on APOE genotype and ischemic stroke outcome to our knowledge. We found a lower age at stroke onset in ε4 carriers and a worse functional outcome in male ε2 carriers. Even larger studies are warranted to further investigate the effects of APOE alleles on ischemic stroke outcome in different age and sex strata.

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P05.40D
Functional characterization of new missense and intronic variants in *LDLR*

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Introduction: About 70% of human plasma cholesterol circulates as a component of low-density-lipoproteins (LDL). Most LDL is cleared from the circulation through the hepatic LDL-receptor(1). Reduction in activity or number of LDLR gives rise to Familial hypercholesterolemia (FH; MIM#143890). Early detection of patients with FH allows initiation of treatment, thus reducing the risk of coronary heart disease. In this study we performed in vitro characterization of new *LDLR* variants found in FH patients.

Materials and Methods: DNA samples from FH patients were analyzed by Next Generation Sequencing (NGS) using a customized panel of 198 genes. The *LDLR* missense variants were generate into the expression vector *LDLR*_NM_000527-Human-cDNA-GFPSpark®-tag by site-directed-mutagenesis and transfected in LDLR-deficient cell line CHO-ldlA7(2,3). Activity and expression of cell surface LDLR were measured by flow cytometry. LDLR expression was detected by Western Blot and immunofluorescence. In order to characterize the *LDLR* intronic variant, the RNA extracted from patient´s peripheral-blood-mononuclear-cells was analyzed by RT-PCR followed by Long-Range-PCR. All transcripts were validated by Sanger sequencing.

Results: Seven new variants at *LDLR* were characterized. The missense variants c.776A>G;p.(Tyr259Cys), c.851G>A;p.(Cys284Tyr), c.1072T>G;p.(Cys358Gly) showed significant differences in activity and expression of LDLR regarding to the wildtype(WT). The variants c.2279C>T;p.(Thr760Ile) and c.2579C>A;p.(Ala860Glu) did not showed any differences. The intronic variant c.2389+4A>G revealed impact on splicing of LDLR resulting as pathogenic.

Conclusions: The functional in vitro characterization of rare variants at the *LDLR* allow us to confirm the genetic diagnosis of FH, avoiding the classification as "uncertain significant variants", and therefore, to allow for cascade family screening.

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P05.41A

Divergent degradation pathways for corresponding LDLR and VLDLR disease-causing mutants

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Introduction: Misfolded proteins in the early secretory pathway are mainly degraded by the ubiquitin-proteasome systems known as ER associated degradation (ERAD) which has been implicated in the pathogenesis of numerous congenital disorders. Therefore, ERAD has been a promising target for therapy of such diseases. In this study we compare and contrast the degradation behavior of equivalent disease-causing mutations in LDLR and VLDLR, two LDLR family receptors.

Materials and Methods: HRD1-SEL1L knockout (KO) HEK293 cell lines have been generated using CRISPR/Cas9. The missense mutants were generated by QuikChange site-directed mutagenesis. The expressed proteins were analyzed biochemically and by microscopy.

Results: We found that the disequilibrium syndrome-causing VLDLR missense mutants (p.D487Y, p.D521H and p.C706F) are retained in the ER whereas only p.D482H and p.C667F of the LDLR corresponding mutants are retained. Two of the ER-retained VLDLR mutants were found to be aggregation-prone leading to ER stress as measured by spliced-XBP mRNA levels. However, there was no differences between LDLR wild type and mutants with regard to their aggregation status or ER stress. Treatment with inhibitors of autophagy seemed to stabilize the degradation of wild type receptors. In addition, p.C706F VLDLR mutant stabilized during early phase of both proteasomal and autophagy inhibition. Cycloheximide chase analysis in SEL1L-KO cells revealed that the HRD1-SEL1L complex could be involved in the clearance of ER-retained VLDLR mutants.

Conclusions: The downstream degradation and ER stress induction behavior of ER-retained corresponding LDLR and VLDLR mutants are divergent suggesting different clearance mechanisms despite their structural similarities.

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P05.42B

Phenotype-driven strategy of DNA diagnostics in patients with LVNC and different types of myocardium remodeling

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Introduction: Left ventricular noncompaction (LVNC) is a cardiomyopathy with causative genetic variants identified in more than 20 genes. It's often accompanied by other types of myocardium remodeling. Several guidelines recommend DNA-diagnostics for LVNC patients but strategy remains unclear. The aim of our study is to evaluate efficiency of DNA-diagnostic in LVNC patients using gene panel (13 candidate genes).

Materials and Methods: We observed 67 probands diagnosed with LVNC. Deep phenotyping was performed including evaluation of cardiac remodeling (dilatation, hypertrophy or isolated noncompaction). Genetic screening by NGS (Ion Torrent) sequencing of 13 genes was performed for all patients. Sanger sequencing of additional genes and whole exome sequencing (WES) were performed if needed. Pathogenecity of genetic variants was analyzed according to ACMG Recommendations (2015). Results. Overall 33 genetic variants in 30 probands (45% of cases) were ascribed to III-V classes of pathogenicity: 12 variants were classified as likely pathogenic/pathogenic, and 21 - as variants of unknown significance. Efficiency of DNA diagnostic was 22.4% in whole cohort but it varied significantly depending on cardiac remodeling. The highest rate of V-IV class variants was found in "LVNC+cardiac hypertrophy" subgroup (53%). In subgroup "LVNC+cardiac dilation" variants of V-IV class were detected in 14% of probands. We detected no mutations in isolated LVNC cases.

Conclusion: Phenotype-driven strategy might be useful for decision-making in LVNC patients. Screening of 13 genes might be cost-effective in "LVNC+cardiac hypertrophy" subgroup. In patients with isolated LVNC/LVNC and dilatation WES might be preferred. This work was supported by RSF grant № 16-15-10421

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P05.43C

Molecular analysis confirmed common ancestor of 10 Czech families with long QT syndrome carrying C926T-*KCNQ1* variant

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Introduction: Long QT syndrome (LQTS) is a hereditary arrhythmic syndrome characterized by abnormal prolongation of QT interval, increased risk of ventricular arrhythmias, and sudden death. It is the most often diagnosed hereditary arrhythmic disorder with prevalence 1:2000. The *KCNQ1* gene is one of the 3 major genes (*KCNQ1*, *KCNH2* and *SCN5A*) which account for 75 % of the genetically identified LQTS cases. The same *KCNQ1* mutation c.926C>T (p.T309I) was identified in 10 putatively unrelated families.

Materials and Methods: 11 highly polymorphic short tandem repeats (STR) markers were chosen for haplotype analysis in 10 families. Multiplex PCR and fragment analysis were performed to identify variant linked to the mutation across families. Single nucleotide polymorphism (SNP) analysis was performed with HumanKaryomap-12 DNA Analysis Kit (Illumina) in one member of each family. 6219 SNPs on p arm of chromosome 11 were analysed.

Results: The same haplotype was identified in the nearest region of the mutation spot in every family by STR analysis and then confirmed by SNP analysis, which also identified possible crossing-overs. The maximum size of the area shared by all families is 658407 bp and contains the whole sequence of *KCNQ1* gene. The maximum size of the area shared by two families is 12633501 bp.

Conclusion: Allelic frequencies of identified alleles in STR markers in control population suggest that there is only one common ancestor with mutation c.926C>T in the group of families investigated in this study.

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P05.44D

Novel *LOX* mutations in five probands with thoracic aortic/arterial aneurysm and dissection with variable connective tissue findings

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Loss-of-function variants in *LOX*, encoding lysyl oxidase, were reported to cause familial thoracic aortic aneurysm and dissection (TAAD). Using gene panel and exome sequencing, we identified five additional probands carrying likely pathogenic *LOX* variants, including three missense variants, affecting highly conserved amino acids and absent from gnomAD. The two stopcodons, (c.(351delC)) and p.(Gly149*), were identified in males with type A dissection at 19 and 51 years without family history. The first proband also suffered from splenic rupture, pneumothorax and varicose veins whereas the second presented with flat feet and inguinal hernia surgery. A p.(Thr99Ala) missense variant was found in a Marfan syndrome-like male with TAA. The second missense variant, p.(Met298Arg), was discovered in 46 year old female presenting with left carotid and prior coronary artery dissection. Interestingly, the identical *LOX* variant previously segregated in five affected members of a TAAD family. The woman presented skin hyperelasticity with recurrent shoulder dislocations. Her mother is known with berry aneurysm, but not available for genetic testing. The last missense variant, p.(Leu306Pro), was found in a tall male with dilatation of the aorta sinus and ascendens with elective surgery at age 15. Family history is significant for aortic dissection at paternal side of the family. Segregation studies revealed the absence of p.(Leu306Pro) in unaffected mother. The latter two missense variants are both located within the LOX catalytic domain.

Our data demonstrate that loss-of-function *LOX* variants cause a wide spectrum of aortic and arterial aneurysmal disease (including coronary artery dissection), combined with connective tissue findings.

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P05.46B
Left ventricular non-compaction and Ebstein anomaly in a patient with a variant in the sarcomere gene MYH7

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Introduction: Left ventricular non-compaction (LVNC) is a rare hereditary cardiomyopathy, resulting from abnormal embryonic myocardial development.

The most prevalent Congenital Heart Disease in LVNC is Ebstein anomaly (EA), characterized by apical displacement and partial fusion of the septal and posterior leaflet of the tricuspid valve with the ventricular septum.

Materials and Methods: We present the case of 46 years old male affected by EA and LVNC.

MR imaging shows an Ebstein anomaly, in which the origin of the septal leaflet is displaced downward into the right ventricle, dividing it into a proximal atrialized and distal ventricularized (true or functional ventricle) chambers. These features, in addition to annular dilatation, result in valve regurgitation and right chambers dilatation. MR imaging shows the coexistence of left ventricle hypertrabeculation with spongy appearance of the myocardium consistent with left ventricular non-compaction.

Genetic counselling was offered. He reported that his nephew was affected by EA and she died at three months of age. Molecular analysis by NGS target panel was performed to our patient.

Results: We identified a heterozygous missense variant (c.728G>A) p.Arg243His in *MYH7* gene, classified as pathogenic according to ACMG Guidelines.

This variant was originally reported in an individual with hypertrophic cardiomyopathy, has subsequently been reported in multiple individuals with LVNC and in an individual with isolated EA.

Conclusions: The association between EA, LVNC and mutations in *MYH7*, seems to represent a subtype of Ebstein anomaly with autosomal dominant inheritance and variable penetrance.

NGS target resequencing represents a valuable tool in cardiomyopathy genetic testing.

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P05.47C
Genetic screening of cardiovascular genes in a heart transplantation cohort

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Heart failure is considered one of the main causes of death in developed countries. Moreover, it is a disease that is influenced by multiple genetic risk factors and the only effective treatment when there are no medical or surgical alternatives would be heart transplant. The aim of this study is to genetically characterized patients whose undergo heart transplantation. We sequenced 65 transplanted patients for a comprehensive cardiovascular gene panel of 209 genes, using Ion semiconductor chips technology in a Ion GeneStudio S5 Sequencer. We identified 29 patients with rare variants (either not described or gnomAD frequency ≤ 0.0001). Twenty-two of them were single carriers (75.9%), six harbor two variants (20.6%), and in one case we identified three variants (3.5%). Regarding to its pathogenicity, eight patients were carriers of at least a variant classified as pathogenic by ClinVar database, six as variants of unknown significance, and the others have no classification. The reason for transplantation of these carriers was due to ischemic heart disease in 48% of cases, 29% to dilated cardiomyopathy, 4% valvular, 4% hypertrophic and 15% to another type of heart disease. In conclusion, we have identified a significant percentage of new or rare genetic variants in genes that would be associated with heart diseases in a heart transplanted cohort. Therefore, detection of these variants might be helpful to achieve an early diagnosis of these diseases. Further co-segregation and functional studies have to be performed in order to accomplish an accurate variant classification.

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P05.48D

Analysis of endothelin-1 (*EDN-1*) UTR regions

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Pulmonary Arterial Hypertension (PAH) is a disease characterized by an increase of secretion and deregulation of Endothelin-1 (ET-1). This peptide is secreted by the endothelium of blood vessels and promotes vasoconstriction. We carried out the characterization of the UTR regions of endothelin-1gene (*EDN-1*), in order to determine common variations that may modulate disease outcome. The analysis was carried out in 60 patients with different classes of PAH, testing a fragment of 2 kb for both UTR region. An *in silico* analysis was performed to evaluate binding transcription factors. Luciferase assay was done to evaluate *in vitro* the SNP influence in gene expression. Data revealed the presence of a deletion in the promoter region (rs397751713), while a transversion in the 3' UTR region was found (rs2859338). The distribution of the genotype frequencies in our PAH patients were: for rs397751713: A/A: 0.08; A/-: 0.27; -/-: 0.66; for rs2859338: A/A: 0.15; A/G: 0.60; G/G: 0.25. Variations are located in a KLF4 binding sequence and a vitamin D receptor binding sequence respectively. Both transcription factors are related to PAH development. In conclusion, these SNPs in the UTR regions of *EDN1* are related with gene expression levels, as we measured higher expression rates for patients with A/A and G/G genotype. Moreover, we hypothesized that this overexpression is due to the inability of KLF4 and vitamin D receptor to attach the target sequence and to regulate the expression of *EDN1*, as KLF4 is probe to avoid PAH when present and vitamin D is an anti-hypertrophic factor.

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P05.49A

Rare *ABCC8* variants identified in Spanish pulmonary arterial hypertension patients

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Introduction: Pulmonary Arterial Hypertension (PAH) is a rare and fatal disease consisting in the obliteration of the pulmonary precapillary arteries, leading to right heart failure and death.

Methods: We used targeted panel sequencing with a custom panel (HAP v1.2) including 21 genes in 318 PAH patients from the Spanish registry (REHAP). After detecting several changes in *ABCC8* we carried out a functional analysis by minigene assay to evaluate possible splicing variants (8/10). Lastly, we used protein modeling by homology (Phyre2) to evaluate the pathogenicity of the changes at protein level assessing its stability with MODELLER.

Results: After validation, we identified ten variants in the *ABCC8* gene that had never been related to PAH: c.298G>A:p.(Glu100Lys), c.2176G>A p.(Ala726Thr) and c.3238G>A:p.(Val1080Ile) were classified as neutral. c.2422C>A:p.(Gln808Lys) and c.3976G>A:p.(Glu1326-Lys) were classified as likely pathogenic. c.1429G>A:p.(Val477Met), c.1643C>T:p.(Thr548Met), c.2694+1G>A, c.3288_3289del:p.(His1097ProfsTer16) and c.3394G>A:p.(Asp1132Asn) were classified as pathogenic. Minigenes confirmed the pathogenicity of c.3394G>A:p.(Asp1132Asn) inducing an exon skipping, and the correct processing of c.298G>A:p.(Glu100Lys) and c.1643C>T:p.(Thr548Met). But they were inconclusive for 5 variants, none of the encoded exons transcribed correctly even in the wild type. Protein modeling of the possible outcomes revealed that amino acid changes would not alter protein stability. The skipping of exons 20, 27 and 32 would yield unstable proteins, while skipping of exon 22 would barely affect stability.

Conclusions: We identified ten variants in *ABCC8*, confirmed experimentally the pathogenicity of c.3394G>A:p.(Asp1132Asn) and bioinformatically c.3288_3289del:p.(His1097ProfsTer16). Protein stability analysis allowed us to predict the possible outcomes of the unconfirmed splicing variants.

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P05.51C
Familial hypercholesterolemia: functional characterization of new variants in *PCSK9*

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Introduction: Proprotein convertase subtilisin/kexin type 9 (*PCSK9*) is a post-transcriptional regulator of the Low-density-lipoprotein receptor(LDLR). "Lost_of_function_-variants" at *PCSK9* has been related with lower LDL-cholesterol while the "gain_of_function_variants" result in autosomal dominant hypercholesterolemia-3 (FH3, OMIN#603776) whose prevalence is 0,1-2%[(1,2)].

Materials and Methods: The DNA samples from patients clinically classified as having probable or definitive familial hypercholesterolemia(FH), were analysed by NGS using a customized panel of 198 genes. The new variants found in *PCSK9* were generated into the expression vector *PCSK9*_NM_174936.3-Human-cDNA-GFPSpark®-tag by site directed mutagenesis. The constructs were transfected in HepG2 cells. We measure both activity and expression of LDLR by flow cytometry in order to determine the impact of *PCSK9* variants.

Results: In silico study showed six variants in *PCSK9:* c.289C>T;p.(Arg97Cys), c.1130C>G;p.(Thr377Ser), c.1495C>T;p.(Arg499Cys), c.1633A>G;p.(Ser545Gly), c.1978G>A;p.(Asp660Asn), c.1987A>G;p.(Thr663Ala) with potential effect in PCSK9. The variant c.1978G>A;p.(Asp660Asn) had a 15% decreased level of internalization of LDL and 20% decreased level of LDLR expression regarding WT-PCSK9. The rest of variants had the same or incremented level of LDL internalization and expression of LDLR regarding WT-PCSK9.

Conclusions: The functional characterization of variants in *PCSK9* has allowed us to classify the variants avoiding the "uncertain significance" variants and thus to confirm the genetic diagnosis of hypercholesterolemia. The results suggest that the variant c.1978G>A;p.(Asp660Asn) could explain the raised level of LDL-c in the patient being a new case of FH3 due to gain-of-function variant in PCSK9.

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P05.52D
Polygenic risk information for coronary artery disease - P5.fi FinHealth

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Introduction: We have tested how polygenic risk scores (PRS) for coronary artery disease (CAD), type 2 diabetes and venous thromboembolism affect the risk of disease within the following ten years in participants from a population-based study FINRISK. We will also utilize NMR metabolomic risk information in disease prevention.

Materials and Methods: Based on follow-up data from national health care registries we modelled PRS in whole genome genotyped population based FINRISK cohorts with multiple registry follow-up for incident cases (N=21726) using Cox regression models. We modelled the impact of genetic and traditional risk factors on a risk of disease within the next 10 years.

Results: We compared the CAD classification of Cox regression model with traditional risk factors and polygenic additive model with 6,6M variants. From basic model risk class 10-20% PRS reclassified 205 participants to highest >20% risk class. In FINRISK participants risk for CAD was higher for men and smoking adds to risk of disease in all PRS classes. Risk for disease increases with BMI being highest in BMI class 30-35.

Conclusions: The validation showed that adding PRS to the traditional risk factors significantly changed the risk enabling reclassification of as many as 17% of the participants. PRS and metabolic risk information are returned to volunteering participants through an internet portal.

Changes in life style are followed up using questionnaires through the portal and the morbidity will be collected through health registers. P5 study is a pilot for P6 which will recruit 200 000 participants and study 10-20 diseases.

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P05.53A

A heterogeneous molecular background of polymorphic ventricular tachycardia in pediatric patients with channelopathy and normal heart

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Introduction: Polymorphic ventricular tachycardia (PVT) is a life-threatening arrhythmia concomitant with the genetic disorders related to myocyte transmembrane ion channel dysfunction called "channelopathies". They comprise long and short QT syndromes (LQTS/SQTS), Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia (CPVT). Although nearly 30 genes have been associated with arrhythmogenic channelopathies so far, the mutation detection rate in syndromes other than LQTS is relatively low (~15-65%), therefore their molecular profile remains largely unknown.

Patients and Results: A 62-gene panel comprising known and candidate genes was used for NGS screening of 12 patients with PVT-associated channelopathy. In 8 patients we identified 4 known and 6 novel, likely pathogenic variants in *KCNH2*, *RYR2* and *SCN5A*. Additionally, rare variants of unknown significance in *AKAP9, KCNA5, KCNH2, KCNT1, MYH6, SCNN1A, TRDN, TTN* were found in 6 cases. Interestingly, in one CPVT patient two novel *RYR2* variants inherited from healthy parents co-occured, suggesting recessive pattern of inheritance. In two other patients diagnosed with PVT and LQTS possibility of digenic inheritance or genetic synergism, associated with changes in *RYR2* and *KCNH2*, has been postulated.

Conclusions: PVT-related channelopathies remain a diagnostic and therapeutic challenge. Phenotypic expression and/or variable penetration observed in the disease may result from the confluence of defects in different genes encoding or modulating ion channels' function. NGS technology provides foundation to better understanding of the genetic profile, phenotype-genotype correlations and etiopathological mechanisms of PVT. Improved detection of risk factors will influence genetic counseling and therapeutic decision-making, preventing severe consequences, like sudden cardiac death.

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P05.54B

RNF213 is a causative gene for pulmonary arterial hypertension and is associated with poor clinical outcomes

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Introduction: Pulmonary arterial hypertension (PAH) is characterized by a strong genetic component. About 30% of patients with idiopathic/heritable PAH have variants in *BMPR2*. The causative genes, if any, in the remaining 70% of patients have yet to be clarified. Since we reported 2 unrelated patients with homozygosity for *RNF213* p. Arg4810Lys who had Moyamoya disease and severe pulmonary hypertension in 2016, we suspected *RNF213* would be causative gene for PAH.

Method: We performed whole-exome sequencing for 76 patients (19 males, 57 females) with idiopathic PAH who had been negative for *BMPR2* and other known pathogenic gene mutations.

Result: We identified *RNF213* p.Arg4810Lys in a heterozygous state in 7 (9.2%) of the 76 PAH patients. Since the minor allele frequency for the p.Arg4810Lys is 0.77% in the normal Japanese population, individuals who are heterozygous for the p.Arg4810Lys might be predisposed to

PAH. From a therapeutic standpoint, patients with the *RNF213* p.Arg4810Lys were low responders to PAH-specific vasodilators. The event-free rate of death or lung transplantation was significantly poorer in *RNF213* p. Arg4810Lys variant carriers than in *BMPR2* variants carriers (5-year event-free rate since the introduction of prostaglandin I2 infusion, 0% vs. 93%, P<0.001).

Conclusion: We demonstrated that nearly 10% of patients with PAH were heterozygous for the *RNF213* p. Arg4810Lys. Documentation of an *RNF213* p.Arg4810Lys might provide clinically relevant information when selecting pharmacologic interventions.

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P05.55C
Customized massive paralleled sequencing panel for diagnosis of Pulmonary Arterial Hypertension

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Introduction: Diagnosis of Pulmonary Arterial Hypertension (PAH) is challenging due to phenotypic overlapping and variable expressivity. Current classification based on clinical features, does not reflect the underlying molecular profiling of these groups, and patients from different groups can share clinical features that could be associated with their response. The advance in the massive paralleled sequencing technologies has allowed describing several new genes related to PAH, improving diagnosis ratio and a better clinical characterization Thus, our aimed was to address the molecular diagnosis of patients with any form PAH

Material and Methods: 318 patients were included in the analysis. 21 gene NGS custom panel was designed the sequencing was performed with a MiSeq. Custom script was developed to annotate and filter the variants.

Results: Pathogenic and likely pathogenic variants were found in 13% of the patients with a 14% of variants of unknown significance. Interesting, we have found variants in patients with connective tissue disease (CTD) and congenital heart disease (CHD). In CTD, we have found one pathogenic mutation and four VUS and, and for CHD two pathogenic variants and 6 VUS. In addition, in a small proportion of patients (0,93%) digenic mode of inheritance was detected.

Conclusions: These results highlight the importance of the genetic screening of PAH and allow to detect variants in PAH-associated forms not described previously. Molecular confirmation of the clinical suspicious is mandatory in cases with clinical overlapping and to perform a properly management and follow up of the patients. Grants **FIS-PI15/02012 Actelion unrestricted grant FCHP-grant**

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P05.57A
A case report of recessive restrictive cardiomyopathy caused by a novel mutation in cardiac troponin I (*TNNI3*)

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Introduction: Restrictive cardiomyopathy is a rare cardiac disease, for which several genes including *TNNT2*, *MYPN*, *FLNC* and *TNNI3* have been associated with its familial form.

Materials and Methods: The index case and her relatives underwent full cardiological assessment. Genetic analysis of the index case was performed using Illumina's Trusight Cardio sequencing panel and Sanger sequencing was used to screen members of the family for the presence of the reported mutation.

Results: Here we describe a female proband with a severely manifested restrictive phenotype leading to heart transplantation at the age of 41 who was found homozygous for the novel *TNNI3* mutation: NM_000363.4:c.586G>C, p.(Asp196His). Her parents were third-degree cousins originating from a small village and although they were found heterozygous for the same variant they displayed no symptoms of the disease. Her older sister who was also

found heterozygous was asymptomatic. Her twin sister and her brother who were homozygous for the same variant displayed a restrictive and a hypertrophic phenotype, respectively.

Conclusion: These observations point to a recessive mode of inheritance reported for the first time for this combination of gene/disease.

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P05.58B
The first Belgian *SCN5A* founder mutation: establishment of an iPSC-cardiomyocyte model to identify genetic modifiers

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Introduction: The *SCN5A* gene encodes the α-subunit of the voltage-gated cardiac sodium channel $Na_v1.5$. Mutations are detected in 20% of the patients with Brugada syndrome (BrS), an inherited cardiac arrhythmia that predisposes to sudden cardiac death (SCD). We have identified a loss-of-function *SCN5A* founder mutation (c.4813+3_4813+6dupGGGT) in 23 different Belgian families. We recruited 89 mutation carriers and their clinical spectrum ranges from completely asymptomatic to palpitations, syncopes and SCD. This provides us with a unique patient cohort to study modifiers that play a role in this variable expressivity and we decided to create patient-specific induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CM) as a model for further investigations.

Materials and Methods: Dermal fibroblasts from a skin biopsy of two patients with different phenotypic severity and two unrelated control individuals were reprogrammed using Sendai viral vectors. The resulting iPSCs were validated using immunostaining, RT-qPCR and embryoid body formation, and for each individual three iPSC clones were established. For differentiation to iPSC-CMs we followed two published protocols and evaluated the cells using immunostaining and patch-clamp experiments.

Results: Optimization of the methods resulted in robust patient and control iPSC-CM models. The patient cells displayed a reduced sodium current compared to the control iPSC-CMs.

Conclusion: We established iPSC-CM models for a unique Belgian *SCN5A* founder mutation displaying remarkable variable expressivity. Further experiments including transcriptomics, whole-genome sequencing, electrophysiological and functional investigations will enable us to identify genetic modifiers and unravel their mechanism of action. This will hopefully stimulate the development of novel drugs for cardiac arrhythmias.

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P05.59C
Clinical and genetic data of 151 individuals from 58 unrelated families with *SMAD3* mutations

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Pathogenic variants in *SMAD3* are mainly responsible for a thoracic aortic disease, characterized by aneurysms and dissections, which has a major impact in terms of morbidity and causing early mortality. These vascular damages are

associated with multisystemic signs including premature osteoarthritis. *SMAD3* encodes one of the canonical TGF-β pathway members whose defect is involved in connective tissue disorders such as Loeys-Dietz syndrome and represents a rare cause (2%) of familial thoracic aneurysms and dissections. Clinical variability and incomplete penetrance are commonly associated with pathogenic *SMAD3* variants. This prompted us to review all the individuals for which a *SMAD3* mutation was identified in our Reference Centre and to compare these with published cases, to determine any genotype-phenotype correlation associated with mutations of this gene. Here, we report clinical and genetic data from 20 new cases from 8 families from our Reference Centre. After a complete review of the literature, we collected a total of 49 unique variants of different nature, from 151 individuals of 58 unrelated families, including missense, truncating and splicing variants. This report confirms the absence of correlation between the mutation type and the phenotype severity and highlights the important inter and intra familial clinical variability and incomplete penetrance described with *SMAD3* mutated patients. Thus, this report brings additional data for the absence of genotype-phenotype correlation of *SMAD3* mutations and the need to explore in more detail the effects of potential modifying factors that could influence the phenotype.

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P05.60D
Clinical utility of *FLNC* variants identified in 3 young sudden cardiac arrest victims/survivors for clinical management of their at-risk relatives

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Introduction: Mutations in *FLNC* gene were initially related to muscular dystrophy and myofibrillar myopathy but more recently, variants in *FLNC* were reported to cause familial cardiomyopathies in the absence of skeletal muscle defects. Truncating mutations in *FLNC* cause an overlapping phenotype of dilated cardiomyopathy or arrhythmogenic cardiomyopathy while missense mutations are associated with familiar hypertrophic cardiomyopathy and might also play an important role in cases of unexplained sudden cardiac death (SCD) in young subjects.

Materials and Methods: We investigated the cohort of Czech cardiac arrest (CA) survivors/victims by performing massively parallel sequencing using a custom-made panel comprising 229 cardiac conditions-related genes (NimbleGen/Illumina). Detected variants were validated by Sanger sequencing, including their familial segregation and classified according to ACMG.

Results: We have identified variants in *FLNC* gene in 3 cases. In 2/3 cases the rare variant NM_001458.4 (FLNC): c.102G>A p.(Trp34*) was found in non-related Czech subjects (26 years old male CA victim and 16 years old female CA survivor). NM_001458.4 (FLNC):c.1732G>T p.(Gly578Cys) in CA victim, where this variant was coexistent with probable causative variant in *TTN* gene.

Conclusion: Both, truncating and missense mutations in the *FLNC* gene might be associated with SCA and demonstrate the clinical utility of genetic testing in at-risk family members. The aim of our study is to increase the usage of genetic analysis in post mortem investigations of SCD in the young cases in order to improve clinical management of relatives at risk in the Czech Republic. Supported by IP00064203/6003; LM2015091; CZ.02.1.01/0.0/0.0/16_013 and AZV - NV18-02-00237.

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P05.61A
Determination of disease-associated genes and gene-sets in Tetralogy of Fallot

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Introduction: Genes and pathways are analyzed for excess of ultra-rare truncating and missense variants in Tetralogy of Fallot using a binomial test comparing observed variation rates to background de-novo mutation rates. This method doesn't require matched controls.

Materials and Methods: Since original background mutation rates were estimated for de-novo variants, we applied a scaling factor to obtain new probabilities P'=k*P; factor k was computed so that the number of predicted and observed ultra-rare variants match. We applied the same method pooling expected probabilities and observed variants by pathway, to boost power. We addressed the problem of gene-set correlations by using a greedy-step-down-aggregation approach; and computed a sampling-based FDR only for aggregated gene-sets. We tested gene-sets derived from Gene Ontology and pathways, and MPO annotation of human orthologs in mouse.

Results: By applying this method to genes predicted haploinsufficient, we found significant genes: FLT4 (BH-FDR~0%), NOTCH1 (BH-FDR~0.5%), and etc. For Gene Ontology and pathways, we found the VEGF and related pathways (FDR~0%, including FLT4,KDR, ⋯), Cardiac Vascular Smooth Muscle Cell Differentiation, and related pathways (FDR~0.08%, including NOTCH1 gene, ⋯). For MPO terms, we found abnormal vitelline vascular remodeling and related pathways (FDR~0%, including FLT4, KDR,FOXO1, ⋯), delayed heart looping and related pathways (FDR~0.05%, including NOTCH1 gene, ⋯).

Conclusions: FLT4,KDR,FOXO1, and NOTCH1 genes were in line with manual gene curation findings. Also, pathways results confirm manual curation findings which support dysregulated VEGF signaling as a novel mechanism contributing to the pathogenesis of TOF. Funded by Ted-Rogers Centre for Heart Research, and CIHR (MOP-89066).

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P05.62B
Pediatric patients with DCM have lower than expected mutation rate in the *TTN* gene

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Introduction: Idiopathic dilated cardiomyopathy (DCM) is has the prevalence of 1:250, and at least one-third of all the cases are inherited. According to various studies focused on the adult patients, mutations in the TT*N* gene accounts 10-30% of DCM cases. The mutation rate in the TTN gene, the characteristics of manifestations and their prognostic significance in childhood have not been studied.

Aim: To determine *TTN* mutation rate in children with DCM and the relevance of including this gene in the DNA diagnostic protocol for pediatric DCM.

Materials and Methods: Complete clinical and instrumental examination of 36 DCM

patients was conducted in cardiology centres. Genetic study had included sequencing based on the IonTorrent ™ platform of the *TTN* isoform N2BA (25 isolated cases) and whole exome sequencing trios on the Illumina platform (11 family cases).

Results and discussion: The group included 36 probands diagnosed with DCM before 18 years (average age: 6.5 years). The sex ratio (M:F) was 23: 8. The only likely pathogenic truncating variant p.Arg33703*in the *TTN* gene was found in a 16-year-old male proband out of 36 (3%). Apparently, *TTN*-dependent forms of DCMs manifest later at a young or more mature age.

Conclusion: These results do not support the TT*N* gene as the first line of DNA diagnostics for DCM in the pediatric group. Further research is needed to compare the representation of mutations in the *TTN* gene in different age groups of DCM patients.

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P05.63C
Large-scale genetic study provides new insights into genetics and etiology of varicose veins

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Introduction: Varicose veins are a common pathology of lower extremities. Although heredity is a well-known risk factor for varicose veins, genetic architecture of this condition is still poorly understood.

Materials and Methods: This study is a genome-wide association study with deep in-silico functional follow-up analysis. Summary statistics for 408,455 European-ancestry individuals were provided by the Gene ATLAS and the Neale Lab projects.

Results: Seven loci associated with the risk of varicose veins were identified that explain 10% of the SNP-based heritability. In six of them, we prioritized the most likely causal genes *CASZ1*, *PIEZO1*, *PPP3R1, EBF1*, *STIM2*, and *HFE*. Gene set enrichment analysis revealed gene categories related to abnormal vasculogenesis. Summary data-based Mendelian Randomization analysis followed by the Heterogeneity in Dependent Instruments test showed pleiotropic effects on body mass-, fat-, blood- and blood pressure-related traits. Genetic correlation analysis confirmed known epidemiological associations between VVs and deep venous thrombosis, weight, rough labor, and standing job, and found a genetic overlap with multiple novel traits, including height, pain, educational attainment, fluid intelligence, walking pace, smoking, and gonarthrosis. Mendelian randomization analysis revealed causal effects of plasma levels of MICB and CD209 proteins and anthropometric traits such as height, weight, waist and hip circumference.

Conclusions: Our results provide novel insight into the genetic underpinnings of varicose veins and their etiology. Prioritized genes and identified proteins can be good candidates for future functional studies. The study was supported by the Russian Science Foundation [Project No 17-75-20223].

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P06
Metabolic and mitochondrial disorders

P06.03B
Farber disease (acid ceramidase deficiency): demographic and diagnostic data from the first-ever natural history study

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Introduction: Farber disease is a rare lysosomal storage disorder caused by mutations in both alleles of the *ASAH1* gene. The resulting deficiency of the lysosomal enzyme acid ceramidase, and accumulation of the pro-inflammatory sphingolipid ceramide, causes a broad spectrum of symptoms and disease severity which may delay diagnosis or lead to misdiagnosis. The ongoing study described here is the first comprehensive, systematic clinical study of the natural history of Farber disease.

Methods: The Observational and Cross-Sectional Cohort Study of the Natural History and Phenotypic Spectrum of Farber Disease (NCT03233841) is designed to collect retrospective and prospective data including demographics, clinical presentation, phenotype, and diagnostic history of patients diagnosed with Farber disease who have or have not undergone hematopoietic stem cell transplantation (HSCT), along with specific prospective clinical evaluations in living patients.

Results: From November 2017 to December 2018, 42 patients (25 living, 17 deceased) have been enrolled in the study. The average age of the living patients is 9 years (range 1 to 28 years). Average time from onset of first symptoms to diagnosis is 2 years (range < 1 to 12 years). Patient countries of birth include Afghanistan, Argentina, Canada, Egypt, Germany, India, Iraq, Italy, Mexico, Sweden, Syria, Turkey, and the USA.

Conclusions: Patients representing the breadth of the phenotypic spectrum of Farber disease, from rapidly progressive (severe), to slowly progressive (attenuated) have

been enrolled from 15 centers (9 countries). Demographic data and numbers of patients enrolled indicate that Farber disease is likely not as rare as previously thought.

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P06.04C

Lysosomal storage, neurodegeneration, and albinism due to effects of a de novo *CLCN7* mutation on lysosomal acidification

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Lysosomes rely on the maintenance of an acidic luminal pH for optimal function. The active accumulation of protons is driven primarily by V-ATPase, but luminal acidification also requires a neutralizing ion movement. Chloride has been proposed to provide this counterion through the transporter ClC-7, encoded by *CLCN7*. Individuals with loss-of-function *CLCN7* mutations develop osteopetrosis; in contrast, here we describe two unrelated children with an identical pathogenic de novo variant in *CLCN7* without osteopetrosis but instead showing a pleotropic syndrome, including cutaneous albinism, developmental delay, hepatosplenomegaly, lysosomal storage, cellular accumulation of large intracellular vacuoles, and no osteopetrosis. The mutation, c.2144A>G; p.Tyr715Cys, increases ClC-7-

mediated chloride flux, decreases lysosomal pH and increases the size and number of intracellular vacuoles in the patients' fibroblasts, with a similar phenotypic spectrum in mice carrying an equivalent mutation. The cellular hyperacidity and cellular phenotype were rescued in vitro by treatment with the alkalinizing agent, chloroquine, emphasizing the role of ClC-7 in regulating lysosomal pH.

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P06.06A
Biallelic disruption of *SDHC* leads to a novel presentation of mitochondrial complex II deficiency

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Introduction: Succinate dehydrogenase complex (i.e. complex II) deficiency is a rare mitochondrial disorder. So far, in three out of the four nuclear encoded proteins composing succinate dehydrogenase (*SDHA*, *SDHB*, and *SDHD*), and in one of its assembly factor genes (*SDHAF1*), biallelic disruption has been shown to cause complex II deficiency with a leukoencephalopathy as central clinical presentation. For the fourth SDH-gene composing succinate dehydrogenase, *SDHC*, biallelic disruption has not been reported, as of yet.

Methods and Results: Here we describe the clinical and molecular analysis of two siblings with biochemically confirmed complex II deficiency, caused by compound heterozygous variants in *SDHC* (c.31C>T; p.(Arg11Cys) and c.202T>C; p.(Ser68Pro)). Segregation analysis supports the recessive pattern of inheritance: in none of four healthy siblings tested both variants were present. One patient had a severe neurological handicap and died of respiratory failure at the age of 11 years. The other sibling was found to be affected at prenatal testing; pregnancy was ended. Interestingly, neuroimaging in the first patient showed progressive cerebral atrophy but not the typical white matter abnormalities seen in previously described complex II deficient patients. This might point towards a distinct feature of complex II deficiency caused by *SDHC* disruption, compared to complex II deficiency caused by disruption of the other SDH-genes and *SDHAF1*.

Conclusion: Here we report for the first time two cases of complex II deficiency caused by biallelic disruption of *SDHC*, emphasizing the importance of screening all SDH-genes in patients with (or suspected of) complex II deficiency.

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P06.07B
Screening & Diagnosis of Congenital Metabolic Disorders using Mass Spectrometry in India: 15 Years' Experience with Issues & Challenges

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Introduction: Most congenital metabolic disorders cause severe pathological sequel, such as mental retardation, sudden infantile death or other irreversible mental/motor disabilities. The dilemma for a clinician is overlapping symptoms in majority of amino, organic & fatty acid disorders like failure to thrive, seizures, vomiting, poor feeding, metabolic acidosis, lethargy, and developmental delay. Hence, a need of metabolic test to cover a large spectrum of disorders in one test.

Materials and Methods: The urinary metabolic profiling to cover more than 140 metabolic abnormalities was developed for screening the 5880 patients. The confirmed diagnosis was by gas chromatography /mass-spectrometry or mutational analysis.

Results: The 48 % (2856 of 5880 cases) were diagnosed to have metabolic abnormality. Amino and organic acidopathies accounted highest as a major cause of mortality and morbidity. Overall, low birth weight (34%), convulsions (33%), premature birth (27%), acidosis, refusal to feed (13%) and respiratory distress (13%) were recorded, with consanguinity, history of mental retardation and death of earlier sibs as other high-risk genetic factors. This high-risk screening indicated that which metabolic disorders are more frequent in Indian population and a need of strategic planning for newborn screening at affordable cost.

Conclusions: The method used for diagnosis covered a large spectrum of metabolic disorders in a shortest time with cost-effective approach & was found significant in a resource constraint Indian scenario. Our experience & data will be demonstrated emphasizing genetic counselling & prenatal mutational diagnosis in few illustrative cases along with issues & challenges while implementation of the study.

U.P. Dave: None.

P06.08C
cDNA structure analysis revealed a novel *CTNS* mutation

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Introduction: Cystinosin, encoded by the *CTNS* gene, is a carrier protein responsible for the export of cystine out of the lysosome. Its deficiency leads to cystinosis, an autosomal recessive lysosomal storage disorder. Here, we report a patient with Infantile Nephropathic type of cystinosis (INC).

Materials and Methods: Total RNAs from peripheral blood mononuclear cells of participants and primary fibroblasts were extracted by the standard Trizol-based method. The cDNA structure analysis was performed by electrophoresis (EP) following Reverse Transcription PCR (RT-PCR) and Sanger sequencing.

Results: The cDNA structure analysis of all exons of the *CTNS* revealed homozygous loss of 4th and 5th exons. On the DNA level we confirmed 9 kb deletion, spanning region from 3rd to 5th introns. In order to identify the inheritance pattern we analyzed the pro-band's mother's CTNS gene both on the cDNA and the DNA levels. Mother was found to be heterozygous for the same mutation.

Conclusions: For INC diagnostics we showed advantages of cDNA sequencing versus DNA sequencing. First of all, in case of some genes, such as *CTNS*, it is cheaper to sequence cDNA fragments rather than all exons separately. We amplified *CTNS* cDNA, consisting of 12 exons,as 3 separate fragments. Secondly, long enough deletions or insertions could be detected by electrophoresis with following confirmation by DNA sequencing. We estimate this novel deletion to be classified as pathogenic according to ACMG criteria because it leads to a truncated CTNS protein.

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P06.09D
D-Bifunctional protein deficiency - diagnosis by whole exome sequencing and fibroblast analysis despite normal plasma biochemistry

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D-Bifunctional protein (DBP) deficiency is an autosomal recessive disorder of peroxisomal fatty-acid beta-oxidation, caused by mutations in the HSD17B4 gene. It is characterised by early-onset neurological symptoms including hypotonia and seizures, developmental delay and eventual neuroregression. Survival beyond 36 months is rare. Diagnosis relies on the detection of accumulated levels of VLCFAs in plasma but occasional patients have undetectable or near normal plasma levels, sometimes associated with longer survival.

We report a 9 year old male patient born to non-consanguineous northern European parents, presenting with neonatal seizures and global developmental delay from early infancy. Developmental progress was made until around 4 years, after which steady neuro-regression was seen. Metabolic testing of urine and plasma, including VLCFAs, demonstrated normal findings on multiple occasions.

Trio based whole exome sequencing undertaken through the DDD study identified compound heterozyosity for a missense and a frameshift variant in HSD17B4, consistent with DBP-deficiency. The diagnosis was confirmed by enzyme analysis in cultured skin fibroblasts which showed reduced DBP activity (mildly reduced hydratase activity; undetectable dehydrogenase activity) despite a persistent normal VLCFA profile, consistent with type III DBP deficiency.

This case highlights the unreliability of plasma VLCFA in the diagnosis of DBP-deficiency, particularly when residual enzymatic activity may be present. Trio based whole exome sequencing is an alternative, with confirmation by analysis of enzyme activity in cultured fibroblasts.

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P06.10A
A case of dihydropyrimidine dehydrogenase deficiency and homozygous mutation DPYD:c.1905+1G>A in Bulgarian patient with severe autistic disorder

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Background: Dihydropyrimidine dehydrogenase (DPD) deficiency is an autosomal recessive disorder of the pyrimidine metabolism characterised by thymine-uraciluria with variable expressivity and different phenotypes. It varies from asymptomatic to severe neurological manifestations and an increased risk of toxicity from 5-fluorouracil treatment.

Materials and Methods: A child with severe autistic behavior, intellectual disability (no speech at 6 years old) and family history - aunt with mental retardation, was referred for genetic counseling. Metabolic screening was performed in urine sample by dipstick - glucose, ketone, pH, and reducing sugars, followed by LC-MS/MS blood aminoacids and acylcarnitines and GC/MS urine organic acids analysis. Mutations in DPYD gene are reported in DPD deficiency patients. The gene was investigated by direct Sanger sequencing in proband and his parents.

Results: Elevated levels of thymine and uracil were detected by GC/MS urine test which was suggestive of DPD deficiency. Molecular analysis of DPYD gene showed a homozygous splice-site mutation c.1905+1G>A in the proband, inherited from heterozygous parents. Subsequently, prenatal testing in the family was performed. The fetus was healthy non-carrier.

Conclusion: Here we report the first Bulgarian patient with severe autistic disorder caused by DPD deficiency and subsequent prenatal diagnostics performed in the family. As patients with this deficiency could be easily detected by determination of the thymine-uraciluria, screening for these defects are indicative for all patients with any autistic behavior and intellectual disability. It also could be useful for those patients who are going to be treated with 5-fluorouracil to prevent lethal toxicities and personalise treatments.

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P06.11B
Electronic nose for diagnosis of metabolic diseases via breath samples

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Inborn errors of metabolism (IEMs) are individually rare but often devastating illnesses characterized by the toxic accumulation of metabolites. Early diagnosis, frequent monitoring, and dietary intervention starting in early infancy substantially decrease morbidity and mortality. However, current diagnosis and monitoring methods rely on repeated blood draws and costly laboratory tests with prolonged turnaround times. Thus, a non-invasive, efficient, and rapid approach is needed. Here, we present a novel approach for monitoring patients with IEMs based on analysis of volatile organic compounds (VOCs) in breath samples using a nanomaterial-based sensor array, the electronic nose (eNose). Exhaled breath was collected from 31 patients with IEMs and 34 healthy controls and analyzed using both eNose and gas chromatography mass spectrometry (GC-MS). IEM patients comprised 21 patients with phenylketonuria (PKU), three patients with ornithine transcarboxylase deficiency, and 7 patients with various other IEMs. Machine learning-based pattern recognition algorithms were used to identify breath profiles that distinguished IEM patients from healthy controls with 78% accuracy, 80-87% sensitivity, and 71-77% specificity. When restricting the analysis to patients with PKU, these patients could be distinguished from healthy controls with 78% accuracy, 80% sensitivity, and 76% specificity. GC-MS showed that dodecane, 5-butylnonane, and a propanoic acid-derived compound were significantly elevated in breath samples from PKU patients. Each of these compounds reliably discriminated PKU patients from controls (c-statistic = 0.786-0.815). Together, these results suggest that breath analysis could become a viable non-invasive clinical monitoring and screening tool for IEMs and other disorders that affect pediatric populations.

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P06.13D
Measurement of myocardial native T1 value in Fabry patients with treatment

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Fabry disease is known as most prevalent lysosomal storage disease, and caused by mutations in the *GLA* gene. Complete or partial defected enzyme activity leads to the accumulation of enzyme substrate, sphingolipids in cells and multiple organ failure. Several reports have been published that native T1 value from magnetic resonance imaging methods may reflects the storage of organs. Several reports also mentioned that native T1 value reduction was observed in patients with Fabry disease which prior of cardial structural or functional changes.

ECG, echocardiographic and MRI, including calculation of native T1 value assessment, were performed with two patients with Fabry disease prior to enzyme replacement treatment. In those patients LVH was absent, but native T1 value showed lower value than means value in others. After starting ERT treatment, no changes in cardiac function were observed. But native T1 values were slightly normalized in after 6-12 month ERT treatment.

The native T1 value is one of the candidate quantitative assessment for myocardial storage for lysosomal diseases. Our results show that it may detect early cardiac involvement and efficacy the treatment of Fabry disease. The reports which mentioned about T1 values in Fabry patients have been limited. Therefore, it is still unclear whether the native T1 value represents myocardial accumurations of substrate in Fabry patients. Further results needed to translate these finding, but native T1 value would be useful for clinical evaluation of lysosomal diseases.

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P06.14A
Molecular heterogeneity in patients with familial partial lipodystrophy

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Introduction: Familial partial lipodystrophy (FPL) is a rare AD transmitted disorder characterized by abnormal distribution of adipose tissue. There are six different types of FLP based on genetic background. The most common form is FPL type 2 (Dunnigan disease) caused by mutation in *LMNA* gene. Typical clinical features of patients with FLP are excessive subcutaneous fat accumulation in the neck and face, reduced subcutaneous fat in the arms and legs and muscular appearance due to muscular hypertrophy. The extent of adipose tissue loss usually determines the severity of the associated metabolic complications such as hyperglycemia, hyperinsulinemia, insulin-resistant diabetes mellitus, increased serum triglicerydes, hepatic steatosis, pancreatitis, high blood pressure, and premature atherosclerosis with an increased risk of coronary heart disease.

Materials and Methods: Among 64 patients tested using custom NGS panel due to familiar dyslipidemia, 9 showed lipodystrophic features. Similarity of the phenotypic traits and characteristic significant hypertriglyceridemia suggested the diagnosis of FPL type 2.

Results: The study revealed the presence of R482Q alteration in *LMNA* gene in 4 subjects in two families and R148K in *PPARG* gene in two subjects in one family.

Conclusions: Recognition of FPL type 2 and type 3 confirm the molecular heterogeneity of familial partial lipodystrophy. Identification of patients with FPL is challenging due to variable and often mild phenotype. Severe hypertriglyceridemia was consistent and easy to identify feature. Thus we recommend all patient with markedly elevated TG to undergo molecular testing for FPL, preferably with targeted NGS. Financing: Statutory Research No.2016/X/9-SZB, PMM Hospital Research Institute

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P06.15B
L-Ferritin: one gene, five diseases; from hereditary hyperferritinemia to hypoferritinemia - Report of new cases

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Introduction: Ferritin is a multimeric protein composed of light (L-ferritin) and heavy (H-ferritin) subunits that binds and stores iron inside the cell. A variety of mutations have been reported in the L-ferritin subunit gene (*FTL* gene) that cause the following five diseases: (1) hereditary hyperferritinemia with cataract syndrome (HHCS), (2) neuroferritinopathy, a subtype of neurodegeneration with brain iron accumulation (NBIA), (3) benign hyperferritinemia, (4) L-ferritin deficiency with autosomal dominant inheritance, and (5) L-ferritin deficiency with autosomal recessive inheritance. Defects in the *FTL* gene lead to abnormally high levels of serum ferritin (hyperferritinemia) in HHCS and benign hyperferritinemia, while low levels (hypoferritinemia) are present in neuroferritinopathy and in autosomal dominant and recessive L-ferritin deficiency.

Methods: We have sequenced FTL gene either by Sanger sequencing or by next generation sequencing (NGS) in three families with abnormal levels of ferritin. Moreover, we have performed an extensive review of all reported variants in the FTL gene linked with the previously described five conditions.

Results: Here, we identified two novel FTL variants that cause dominant L-ferritin deficiency and HHCS (c.375+2T > A and 36_42delCAACAGT, respectively), and one previously reported variant (Met1Val) that causes dominant L-ferritin deficiency.

Conclusions: Globally, genetic changes in the FTL gene are responsible for multiple phenotypes and an accurate diagnosis is useful for appropriate treatment. We included a diagnostic algorithm for the detection of diseases caused by defects in FTL gene.

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P06.16C
The contribution of molecular genetic methods to the diagnosis of classical galactosemia and investigation of genotype-phenotype correlation

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Introduction: Classical galactosemia is a disorder of pathogenic *GALT* variants leading to galactose-1-phosphate-uridyl-transferase deficiency. Majority of the pathogenic variants are small sequence alterations, nevertheless gross alterations have also been reported (1%). We aimed to investigate the frequency and distribution of *GALT* mutations in Turkish galactosemia patients with genotype-phenotype correlation.

Material and Methods: Clinically and biochemically diagnosed 91 patients with galactosemia were included in this study. Algorithmic genetic testing strategy for *GALT* was performed initially with Sanger sequencing (n=91) followed by MLPA (n=19), only for cases with incompatible sequence result.

Results: We identified 11 known and four novel pathogenic variants (p.R67Pfs*19, p.S236Rfs*30, p.S156*, p.V243I) in 91 patients leading to 95% detection rate. Retrospective investigation of mutation unidentified cases were tentatively considered galactosemia initially however deviated in follow ups.

Conclusions: As in European cohorts, p.Q188R in exon 6 was the most common (38%) *GALT* mutations. Second most frequent mutation was p.E340* (14%) in exon 10, which is known to be specific for population in Turkey. The third most frequent mutation (8%) was a novel single nucleotide deletion leading to frameshift (p. R67Pfs*19) in exon 2, found to aggregate in patients from eastern Turkey. Algorithmic analysis of exons 6 and 10 would delineate molecular genetic diagnosis for 60% of the patients. Absence of gross mutations is attributed to our small sample size. Nevertheless, MLPA should still be recommended for galactosemia patients with unidentified pathogenic sequence variants in *GALT*.

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P06.17D
GBA1 mutational spectrum in the Republic of Macedonia and a report of a novel *de novo* mutation

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Introduction: Gaucher disease (GD) is caused by the deficient activity of β-glucocerebrosidase due to homozygous or double heterozygous *GBA1* mutations. In addition, heterozygous *GBA1* mutations represent the most common genetic risk factor for Parkinson's disease. We aimed to determine the *GBA1* mutational spectrum among GD patients and the frequency of common *GBA1* mutations among the general population from R. Macedonia.

Material and Methods: Thirty-four individuals belonging to 10 families with 15 GD Type 1 patients, as well as 392 individuals from the general population were studied. GD patients were studied using several different methods: direct DNA sequencing of *GBA1* exons, Multiplex Ligation Probe-Dependent Amplification (MLPA), and next-generation sequencing on MiSeq using Illumina TruSight Inherited panel. Allele-specific amplification was designed to screen for five common *GBA1* mutations among the general population.

Results: A total of eight pathogenic variants (c.115 +1G>A, c.392A>G, c.882T>G, c.1226A>G, c.1263-1317del, c.1312G>A, c.1342G>C and c.1363A>G) were detected. The most common mutation was c.1226A>G (N370S), representing 60% of *GBA1* alleles. The c.882T>G and c.1342G>C were present on one allele (H255Q: D409H). The novel c.392A>G variant was detected in one GD patient as a *de novo* event. The frequencies of *GBA1* mutations among the general population were the following: c.1226A>G (3/700, 0.43%), c.882T>G/c.1342G>C (2/784, 0.26%), c.115+1G>A (1/668, 0.15%), while c.1263-1317del was not detected among the studied individuals.

Conclusion: The knowledge of *GBA1* mutational spectrum in our country will allow for better management of GD patients as well as easier carrier screening among GD family members and Parkinson's patients.

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P06.18A

FPLD3-associated PPARγ mutants define subclasses of target genes

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Introduction: The nuclear receptor PPARγ, encoded by *PPARG*, is the master regulator of adipocyte differentiation and function. Mutations in *PPARG* cause familial partial lipodystrophy subtype 3 (FPLD3), characterized by adipose tissue redistribution causing type 2 diabetes, and dyslipidemia. In two non-consanguineous patients we identified PPARγ E379K and R212Q. The mutations are situated in distinct domains of the PPARγ. Whereas, E379K is located in the ligand binding domain (LBD) and contacts the DNA binding domain (DBD) of its binding partner RXRα in the PPARγ-RXRα-DNA crystal structure, R212Q is located in the hinge region and interacts with the DNA. Methods and materials: We have used genome-wide sequencing-based strategies in *PPARG* knockout mouse embryonic fibroblasts transduced with PPARγ to determine how these *PPARG* mutations - that do not interfere in interactions with RXRα, ligand, and cofactors, but do impair the adipogenic capacity - affect the ability of PPARγ to activate *PPARG* target genes in the context of native chromatin structure.

Results: Both *PPARG* mutations impair the transactivation potential of PPARγ on an overlapping subset of target genes. Classical PPARγ target genes such as *FABP4*, *ANGPTL4*, and *LPL* are among the genes that are highly affected. Genome-wide profiling of PPARγ (WT and mutants) and the enhancer activity marker H3K27Ac shows that these *PPARG* mutations impair PPARγ binding on enhancers that require PPARγ for chromatin remodeling.

Conclusion: These findings indicate that a subset of PPARγ target genes are sensitive to *PPARG* mutations. In addition, relatively subtle molecular defects in PPARγ are sufficient to cause lipodystrophy.

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P06.19B

Ten novel mutations in the mitochondrial elongation translation factor EFG1 are associated to neurological involvement

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Mutations in nuclear genes encoding co-factors or structural subunits of the mitochondrial translation machinery prevent the synthesis of the 13 mitochondrial DNA encoded proteins which causes OXPHOS deficiency. Mutations in the GFM1 gene, encoding the mitochondrial translation elongation factor EFG1, have been linked to mitochondrial diseases with neurologic or hepatic presentations in few patients.

Here, we further expand the genetic and phenotypic spectrum of GFM1-linked disease mutations by reporting clinical, biochemical and neuroimaging findings from 9 unrelated pediatric patients harboring homozygous or compound heterozygous GFM1 mutations, including 10 novel pathogenic variants.

All patients were born at term and without fetal distress. In 8/9 children, the firsts clinical signs were noticed during the neonatal period. Only 1 child had psychomotor regression and lactic acidosis (8 months) after initial normal development. All patients presented with neurological involvement with axial hypotonia and dystonia being the major signs. West syndrome was frequently observed (5/9 patients). One child had liver failure. Western blot analysis on patient fibroblasts revealed that all mutations result in decreased abundance of the mutant EFG1 protein. Specific decrease of mtDNA-encoded proteins confirmed that mitochondrial translation was impaired in patients, which in turn, resulted in an impaired biogenesis of OXPHOS complexes I and IV.

In conclusion, GFM1 mutations result in OXPHOS deficiency and constant neurological involvement with hypotonia, dystonia, and epilepsy. Some patients had a neonatal rapidly progressive disease, but a stable course is more frequent. Liver failure is not a constant feature of GFM1 related diseases.

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P06.20C

Mutations in *GLDC* and *AMT* genes in Czech and Slovak patients with nonketotic hyperglycinemia

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Introduction: Nonketotic hyperglycinemia (NKH, MIM#605899) is a rare, life threatening disorder caused by a defect in the mitochondrial enzyme complex - glycine cleavage system (GCS). The defect results in an accumulation of glycine in the body. Classical form of NKH presents in the first days of life with lethargy, hypotonia, seizures, coma and apnea. Surviving patients have profound developmental delay and severe, usually pharmacoresistant epilepsy. Cerebral spinal fluid (CSF) and plasma glycine levels are elevated as well as CSF:plasma glycine ratio. NKH is an autosomal recessive disorder with causative mutations identified in genes encoding the components of GCS - *GLDC*, *AMT* and *GCSH*. We present the results of mutation analysis in seven patients with classical form of NKH.

Materials and Methods: Analysis of the *GLDC* and *AMT* genes was performed by Sanger sequencing and MLPA.

Results: Mutations in the *GLDC* gene, including a large deletion of exons 10 to 25, were detected in four patients. Five mutations (c.1420G>A, c.1543A>G, c.1877T>G, c.2594del, c.2579G>T) are novel. Mutations in the *AMT* gene were identified in three patients. Analysis of mutations in parents was performed to confirm compound heterozygosity in all patients.

Conclusions: Identification of molecular defect enabled genetic counseling and prenatal genetic testing in affected families. We report 5 novel *GLDC* mutations and our findings expand the spectrum of described variants associated with NKH.

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P06.21D

Genetic analysis of patients with GLUT1DS suspect: the importance of diagnostic confirmation

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Introduction: Classic patients with GLUT1DS present hypoglycorrhachia, drug-resistant epilepsy, developmental delay, a complex movement disorder and, in 50% of the cases, an acquired microcephaly. Furthermore, patients respond to ketogenic diet.

Methods: In this study, 48 suspected cases of GLUT1DS were included. Inclusion criteria were: CSF glucose level below 50.5 mg/dL and clinical findings suitable for GLUT1DS. We have performed the identification of exonic SNV and deletions in *SLC2A1* by Sanger combined with MLPA or its entire sequence were analysed by next generation sequencing (NGS). Patients without pathogenic variants in *SLC2A1* were analysed by TruSight One® panel (Illumina®).

Results: We have detected 27 patients with variations in *SLC2A1*. Ten of them were *de novo* mutations, while five others were associated to maternal or paternal inheritance (missense variants). The mutational spectrum in *SLC2A1* includes two large deletions, three small deletions, one small duplication and twenty nucleotide changes (eighteen likely missense and two splice site mutations). Fifteen variants are novel, five loss-of-functions and ten likely missense, six with unknown significance following the ACMG guidelines. In twenty-one cases no pathogenic mutations in *SLC2A1* were detected and were further analysed by TruSight One® panel, allowing the detection of pathogenic variants in eight different genes related to ion channels, transcriptional factors or cellular trafficking.

Conclusions: Our results show that a response to ketogenic diet, drug-resistant epilepsy and/or hypoglycorrhachia is not a pathognomonic marker for GLUT1DS. Moreover, the results highlight the fact that genetic analysis should be a must-have for GLUT1DS classification.

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P06.22A

Targeted next generation sequencing in selected inborn errors of metabolism

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Introduction: We implemented a targeted NGS for the diagnosis of patients with a suspicion for selected groups of inborn errors of metabolism (IEM).

Methods: A total of 195 genes were analyzed using a custom-designed oligo capture probe set (Roche NimbleGen) and MiSeq sequencer (Illumina). Positive results were confirmed by Sanger sequencing.

Results: In total, we identified 74 mutations in genes associated with IEM. The found mutations were usually SNVs or small deletions/insertions. In two cases we identified gross deletions (*PHKA2*, NM_000292.2: deletion of exon 33, *AGL*, NM_000642.3: deletion of exons 11-21). Genes associated with glycogen metabolism were analyzed in 46 probands. The diagnosis of GSD was confirmed by finding of causative mutations in 19 of them: *GYS2* (1x), *G6PC* (1x), *AGL* (4x), *PYGL* (2x) and *PHKA2* (11x). We established the diagnosis in 3 out of 6 patients with rhabdomyolyses (2x *RYR1*, 1x *PFKM*). Four out of 6 patients were diagnosed in the group of peroxisomal disorders (1x *PEX1*, 1x *PEX12*, 2x *HSD17B4*). Among 17 patients with hyperhomocysteinemia we identified 1 patient with CblD deficiency (*MMADHC*), 2 patients with FTCD deficiency, and 3 patients with the MTHFR deficiency. In two patients with biochemical findings leading to urea cycle disorders we diagnosed 1 patient with argininosuccinic aciduria (*ASL*) and 1 patient with CPSI deficiency. The diagnostic yield for MSUD was 100% (1x *BCKDHA*, 8x *BCKDHB*).

Conclusions: The correlation of phenotypic signs, biochemical findings and NGS results is crucial for completing the diagnosis of IEM. Support: MZ CR - RVO VFN64165, SVV No.260367

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P06.23B

Haemochromatosis type 4: structure/function analysis of the newly identified p.Ser47Phe missense mutation provides new insights about the ferroportin biology and mechanisms of disease

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Haemochromatosis type 4 is the second most frequent cause of inherited iron overload after *HFE*-related haemochromatosis. The disease is due to heterozygous mutations in the *SLC40A1* gene, which encodes the sole iron export protein reported in mammals; namely ferroportin 1 (FPN1). A large phenotypic heterogeneity is observed in adult patients. This is partially explained by the existence of two categories of genetic variants: loss-of-function mutations, which lead to a progressive accumulation of iron in reticuloendothelial cells, and gain-of-function mutations, which are responsible for a partial to complete resistance to hepcidin (the systemic iron metabolism regulatory hormone) and a progressive iron deposition in parenchymal cells. The p.Ser47Phe missense mutation was identified in 8 related patients presenting with an iron overload phenotype characteristic of FPN1 dysfunction. *In vitro* evaluations were performed in HEK293T cells. They revealed that the 47Phe mutant was correctly expressed at the cell surface, while it significantly reduced ability of FPN1 to export iron. They also evidenced that the 47Phe mutant was strongly resistant to hepcidin. Using a 3D model of human FPN1, built by homology to the experimental structure of a bacterial homolog (BbFpn) in the outward-facing state, we observed that Ser47 is located in the vicinity of residues that play a critical role in the transport of iron across the plasma membrane. The region might also be involved in the docking of hepcidin and down-regulation of FPN1. We conclude that p.Ser47Phe missense mutation is responsible for ambivalent functional effects, with loss-of-function having a predominant consequence on phenotype.

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P06.24C
Polygenic risk score profiling of quantitative lipid traits: lessons from a specialized dyslipidemic cohort

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Introduction: Polygenic risk scores (PRSs) are often calculated in prospective cohorts representing generally healthy populations. Given the rarity of phenotypic extremes, characterizing the polygenic underpinnings of a quantitative trait's full spectrum can be challenging. In patients referred to a specialized lipid clinic, we aimed to establish polygenic profiles for triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).

Materials and Methods: We performed PRS analyses using a 16-SNP TG score (N=1,406), 9-SNP HDL-C score (N=1,298), and 10-SNP LDL-C score (N=1,226). For clinical practicality, we utilized smaller PRSs that incorporate SNPs with the largest phenotypic effects. Extreme scores reflecting an excess of trait-raising SNPs were defined as scores ≥90th percentile, as calculated in the 1000 Genomes cohort.

Results: As TG levels increased, the prevalence of extreme TG PRSs greatly increased; patients with the highest TG levels were 5.30-fold ($P<0.0001$) more likely to have an extreme PRS compared to those with normal levels. As HDL-C levels increased, the prevalence of extreme HDL-C PRSs increased slightly; patients with the highest HDL-C levels were 2.37-fold ($P=0.003$) more likely to have an extreme PRS compared to those with normal levels. As LDL-C levels increased, the prevalence of extreme LDL-C PRSs increased slightly; however, the prevalence of extreme PRSs was not different between patients with the highest LDL-C levels and those with normal levels.

Conclusions: The polygenic profiles of these lipid traits were quite distinct. Evaluation of patients at phenotypic extremes allows for more efficient use of resources to define clinically relevant genetic determinants.

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P06.25D
Hyperphenylalaninemia: identification and characterization of pathogenic variants in *DNAJC12*

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Introduction: Hyperphenylalaninemia (HPA) is caused in 98% of cases by loss-of-function mutations in the gene coding for the hepatic protein phenylalanine hydroxylase (PAH). The remaining 2% accounts for defects in the synthesis or regeneration of tetrahydrobiopterin (BH4), cofactor of PAH and tyrosine and tryptophan hydroxylases (TH, TPH), both neurologically expressed. Biallelic variants in gene *DNAJC12*, cochaperone of these hydroxylases, were recently described in HPA patients with neurological symptoms and early-onset Parkinson. Here, we report the genetic analysis of *DNAJC12* in unsolved Spanish HPA cases and the functional characterization of variants.

Patients and Methods: The study included *DNAJC12* sequencing of 15 Spanish HPA cases (120-442 μM) neurologically asymptomatic. BH4 treatment was only required under fever episodes. Functional analysis of variants was done using patient-derived fibroblasts transiently transfected with the three hydroxylases.

Results: Four novel nucleotide changes were identified in *DNAJC12* in 15 HPA cases: two pathogenic variants probably affecting splicing (c.298-2A>C, c.502+1G>C) and two likely pathogenic variants c.309G>T (p.Trp103Cys) and c.524G>A (p.Trp175Ter). The change p.Trp175Ter, present in 0.1% of Spanish control population, was detected in 80% of alleles, with 9 homozygous cases. *DNAJC12* mRNA levels were slightly diminished and immunorreactive protein was undetectable. PAH transfection suggest a pathogenic effect on PAH and TH stability while no effect was found for TPH.

Conclusions: *DNAJC12* sequencing should be incorporated in routine HPA genetic confirmation of cases detected in newborn screening to apply a tailored therapy. To avoid future neurological complications, our cases should be included in clinical follow-up.

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P06.26A
Mutations in SURF1 Gene Associated with Leigh Syndrome İn Turkey

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Introduction: Leigh syndrome (LS) associated with cytochrome *c* oxidase (COX) deficiency is an early onset, fatal mitochondrial encephalopathy, leading to multiple neurological failure and eventually death, usually in the first decade of life. Mutations in *SURF1*, a nuclear gene encoding a mitochondrial protein involved in COX assembly, are the most common cause of LS.

Material-Methods: *SURF1* gene was sequenced in 48 patients with clinical suspicion of mitochondrial disorder (mainly complex IV deficiency and LS) between January 2016 and January 2019. Demographical, clinical and molecular findings of the patients were obtained from hospital files.

Results: We identified 13 patients with clinical features of LS who are either homozygous or compound heterozygous for *SURF1* mutations. Of the 26 mutant alleles, 14 (54%) had null mutations (8 frameshift and 6 nonsense) 12 (46%) had missense. The most common two mutations were C.769G>A (6/26) and c.870dupT (6/26) (%23). Although mutations in *SURF1* have been mainly associated with typical LS, three of the patients in this report had an atypical course of LS. There is no definite genotype-phenotype correlation. Electron transport chain studies were performed in six patients in whom either muscle or skin fibroblasts specimen available. The COX activity in each of these six patients was significantly decreased.

Conclusions: To date, more than 100 patients of Leigh disease with SURF1 mutations have been reported. We report clinical and molecular findings of 13 patients from 12 families and also the first case series of *SURF1* gene deficiency from Turkey.

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P06.27B
Progeroid syndrome with focal segmental glomeruloscrelosis due to *ZMPSTE24* mutations

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Introduction: Biallelic ZMPSTE24 mutations cause mandibuloacral dysplasia with type B lipodystrophy (MADB), an autosomal recessive progeroid syndrome characterized by mandibular hypoplasia, progressive acroosteolysis, and lipodystrophy. Two MADB patients with focal segmental glomerulosclerosis (FSGS) were reported previously. Herein, we present an additional MADB patient with FSGS due to *ZMPSTE24* mutations.

Materials and Methods: The patient is 42-year-old Japanese female. Her older sister had succumbed from diabetic nephropathy at age 41 years. Her clinical course was as follows: subcutaneous fat atrophy since childhood; proteinuria in her twenties; proteinuria with hypertension after delivering her son at age 31 years; diabetes mellitus (DM), hyperlipidemia, and fatty liver at 36 years; loss of visceral fat and renal dysfunction due to FSGS proven on kidney biopsy at 39 years, and kidney transplantation at 41 years. At age 42 years, she was referred for diagnostic work up. She showed a progeroid appearance with decreased subcutaneous fat. Radiographs revealed open cranial suture and thin clavicles. The study protocol was approved by the Institutional Review Boards of Tokyo Women's Medical University. Written informed consent was obtained.

Results: Two rare heterozygous variants, p.Q41X and p.R412L in *ZMPSTE24*, were detected. R412L, a novel variant conserved over species, was not found in ExAC or 1000G, and was predicted to be pathogenic on PolyPhen2 and Mutation T@ster. She has since received Metreleptin injections, achieving improvements of DM, liver dysfunction, and renal dysfunction.

Conclusion: We reported a progeroid patient associated with FSGS caused by *ZMPSTE24* mutations. Metreleptin was estimated to ameliorate renal function.

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P06.28C
Isolated methylmalonic acidaemia in Slovakia

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Isolated methylmalonic acidaemia (iMMA) is a group of autosomal recessive disorders caused by impairment of propionate metabolism. The severity of clinical presentation depends on the underlying gene and can range from mild to life-threatening condition with failure to thrive, hypotonia, metabolic acidosis, developmental delay, encephalopathy, pancreatitis, and chronic kidney disease. The common sign is increased methylmalonic acid plasma/urine level and hyperamonaemia. iMMA is genetically heterogeneous and can be due to defects in genes involved in propionate pathway or cobalamin metabolism. The most common are *MUT*, *MMAA*, and *MMAB*.

Patients and Methods: Nine children were reported for genetic testing. Five patients were clinically diagnosed after manifestation before year 2013 and 4 patients were found in extended neonatal screening from blood spots that has been undertaken in Slovakia since 2013. Genes *MUT* and *MMAA* were sequenced using Sanger sequencing. One patient was analyzed using whole exome sequencing.

Results: The diagnosis of iMMA was genetically confirmed in all tested patients. Six patients had bi-allelic *MMAA* mutations (three different known pathogenic variants and one novel variant p.A102T), two patients had bi-allelic *MUT* mutations (two known pathogenic variants and one novel variant p.R93C) and one patient had a homozygous known pathogenic *MMAB* variant.

Conclusions: The neonatal screening from blood spots is an effective way for early pick-up of individuals with iMMA. The most prevalent iMMA underlying gene in Slovakia is *MMAA* followed by *MUT* and *MMAB*, unlike reported in other countries, where the *MUT* gene is the most prevalent one. Support: APVV-17-0296, VEGA 2/0083/17, APVV-107-12

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P06.30A
A novel homozygous missense mutation in *TUFM* leads to a mitochondrial cardiomyopathy syndrome without progressive encephalopathy

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Introduction: Mitochondrial dysfunction typically results in clinically heterogeneous, often devastating, disorders. Accurate molecular diagnosis can be challenging. We describe a proband, the third son of healthy consanguineous parents of Muslim Arab descent. He presented at six months with fatal, severe cardiogenic shock, dilated cardiomyopathy, lactic acidosis and multiple mitochondrial respiratory complex deficiency.

Materials and Methods: We performed trio whole-exome sequencing (WES) and singelton mitochondrial genome sequencing. Results were filtered for rare, protein-altering variants in genes related to mitochondrial function. Protein structures were modelled using SWISS-MODEL based on the bovine crystal structure of mitochondrial Elongation Factor Tu/Ts complex (PDB #1XB2), and visualized with UCSF Chimera software.

Results: We identified a novel homozygous missense variant in *TUFM* (NM_003321.4): c.344A>C; p.His115Pro, encoding the mtDNA translation elongating factor Tu (EFTu). Pathogenic variants were not detected in mitochondrial DNA.

This missense variant is located in domain 1 of the EFTu protein, which is required for GTP/GDP-binding and for complex formation with elongation factor Ts (EFTs). This highly conserved His115 (GERP 5.42) is predicted to be an exposed and functional residue; therefore, substitution with a physiochemically different residue may disrupt proper protein folding, and may hamper Tu/Ts complex stability and EFTu reactivation.

Conclusions: To date, only four patients with bi-allelic *TUFM* mutations have been reported, all with severe early-onset lactic acidosis and progressive infantile encephalopathy without cardiomyopathy. We present a novel variant in a patient with dilated cardiomyopathy and lactic acidosis but without early encephalopathy, thus expanding the phenotype of *TUFM*-related mitochondrial disease.

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P06.31B

Efficacy of WES in patients with "unsolved phenotype": expansion of the phenotype spectrum associated with mutations in *KARS* gene

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We evaluated a 14-month-old girl with apostural tetraparesis, growth delay, severe psychomotor retardation, profound sensorineural hearing loss, leopard-like retinopathy, hypogammaglobulinemia, anemia and thrombocytopenia, hepatopathy, esophageal varices and calcifications in the hepatic segment VI, startle-type critical manifestations, brain calcifications, progressive cortico-subcortical atrophy that result in tetraventricular hydrocephalus. Moreover, reduction of mitochondrial respiratory complex-I-III was observed. The serious conditions led the patient to exitus at 24 months of age. Several molecular tests (panel genes for brain calcification, Aicardi-Goutières syndrome, disorders of glycosylation, peroxisomal disease) and CGH-array were normal. Whole exome sequencing (WES) identified two rare variants in *KARS* gene. *KARS* gene encodes the mitochondrial and cytoplasmic isoform of the t-RNA synthase of lysine, essential for a correct protein synthesis. To date, mutations in *KARS* are associated with autosomal recessive Charcot-Marie-Tooth, non-syndromic hearing loss, clinical features characterized by microcephaly, epilepsy, leukoencephalopathy, peripheral neuropathy, visual impairment, auditory and hepatic failure and others with severe cardiomyopathy, psychomotor and mild delay myopathy. Functional study on muscle biopsy of our patient revealed an alteration of protein expression.

Our case shows once again how WES analysis is the first level method in clinical practice in children with multi-systemic, neurological and/or neuromuscular problems, mostly in case of mitochondrial disorders whose diagnosis is often problematic because of their large phenotypic and genotypic heterogeneity. Finally, the use of WES allowed us to outline a new phenotype associated with a poorly characterized gene.

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P06.32C

Muscle involvement in a large cohort of pediatric patients with genetic diagnosis of mitochondrial disease

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Mitochondrial diseases (MD) are a group of genetic and acquired disorders which present significant diagnostic challenges. Here we report the disease characteristics of a large cohort of pediatric MD patients ($n = 95$) with a definitive genetic diagnosis, giving special emphasis on clinical muscle involvement, biochemical and histopathological features. Of the whole cohort, 51 patients harbored mutations in nuclear DNA (nDNA) genes and 44 patients had mutations in mitochondrial DNA (mtDNA) genes. The nDNA patients were more likely to have a reduction in muscle fiber succinate dehydrogenase (SDH) stains and in SDH-positive blood vessels, while a higher frequency of mtDNA patients had ragged red (RRF) and blue fibers. The presence of positive histopathological features was associated with ophthalmoplegia, myopathic facies, weakness and exercise intolerance. In 17 patients younger than two years of age, RRF and blue fibers were observed only in one case, six cases presented cytochrome c oxidase (COX) reduction/COX-fibers, SDH reduction was observed in five and all except one presented SDH-positive blood vessels. In conclusion, muscle involvement was a frequent finding in our series of MD patients, especially in those harboring mutations in mtDNA genes.

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P06.33D

Multiple mitochondrial DNA deletions due to mtDNA-maintenance defects are identified by next generation sequencing of the mitochondrial genome through eKLIPse

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Purpose: Diseases caused by nuclear genes affecting mitochondrial DNA (mtDNA) stability are an important group of mitochondrial disorders and characterized by multiple mtDNA deletions. However, mtDNA deletions also accumulate during aging, making their interpretation difficult in patients suspected of mitochondrial diseases. The objective of this work was to identify criteria to distinguish normal aging *vs* mtDNA maintenance defects.

Methods: MtDNA of patient muscles with known pathogenic variants in nuclear genes involved in mtDNA maintenance (n=34) and muscle from controls without mitochondrial enzyme and assembly defects (n=53) were sequenced by next-generation sequencing (NGS). The sequencing data were processed through eKLIPse, a bioinformatic tool allowing the detection of mtDNA rearrangements. The deletions' profiles were compared between patients and controls according to age groups.

Results: In the overall cohort, selected parameters such as location or number of deletions and heteroplasmy level were significantly different between patients and controls. However, the criteria for identifying mtDNA maintenance

defects was depending on patient age: The number of deletions appearing to be significantly relevant only in the elderly, while in young subjects breakpoint repeat lengths surrounding the deletions were discriminant.

Conclusion: EKLIPse analysis of mtDNA NGS allowed to discriminate age-related mtDNA rearrangements from those related to mtDNA maintenance defects. These characteristics are promising criteria in order to guide the molecular diagnosis towards mtDNA maintenance defects and also to facilitate the prioritization of novel variants identified in nuclear-encoded genes involved in mtDNA stability.

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P06.34A

Molecular diagnosis of mitochondrial respiratory chain complex deficiencies by whole exome sequencing

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Mitochondrial disorders are considered to be rare diseases which are genetically heterogenous. The oxidative phosphorylation system consists of five multiprotein enzyme complexes. We report here two cases of patients suspected to have a mitochondrial disorder whose samples were banked at the Malta BioBank (BBMRI.mt).

The analysis was part of a collaborative BBMRI-Large Prospective Cohort (BBMRI-LPC) project focused on mitochondrial disorders. The full mitochondrial genome sequenicng, whole exome sequencing (WES) and data processing were carried out at Centro Nacional de Análisis Genómico (CNAG-CRG). Phenotypic data was recorded in the RD-Connect PhenoTips instance, and variant filtration and prioritisation was undertaken using the RD-Connect Genome-Phenome Analysis Platform.

In one patient WES identified a rare nuclear homozygous mis-sense variant c.308C>T (rs749249430) in ***NDUFAF3*** on chromosome 3, three INDELs in ***NDUFS1***, intronic variants in ***NDUFA10*** on chromosome 2 and a heterozygous intronic variant c.408+6468C>T (rs752756523) in ***NDUFB9*** on chromosome 8. In the other patient, a mitochondrial DNA (mtDNA) mis-sense mutation in ***MT-ATP6*** c.163A>G at m.8689 and a splice donor variant c.207 +2T>G (rs782792601) and two mis-sense splice region variants: c.206A>G (rs781909386) and c.205A>G (rs782503581) in ***NDUFB11*** were identified on the X chromosome.

NDUFAF3 is a mitochondrial respiratory chain complex I (C1) assembly factor. mtDNA mutation m.8689 is associated with mitochondrial complex V deficiency and Leigh syndrome while NDUFB11 is associated with mitochondrial C1 deficiency. The pathogenicity of these genes is in the process of being confirmed in patient fibroblasts.

WES followed by functional validation of disease alleles could identify disease-causative variants in mitochondrial respiratory chain complexes.

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P06.36C

Two long-surviving adults with Myopathy Lactic Acidosis Sideroblastic Anemia 1 (MLASA1) due to previously undescribed mutations in *PUS1*

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Introduction: Myopathy, lactic acidosis, and sideroblastic anemia 1 (MLASA 1, MIM#600462) is a rare, autosomal recessive mitochondrial disease which is most often fatal by adolescence. A few cases of long-surviving patients have been reported. Microcephaly, short stature, cognitive impairment and cardiomyopathy are variable features. We present two females aged 26 and 31, further demonstrating long survival in MLASA 1.

Materials and Methods: Patient 1 presented with myopathy, lactacidosis and anemia at age 7, and MLASA 1 was suspected. Patient 2 presented with anemia at age 24. Severe exercise intolerance and myopathy since childhood was revealed. In both patients *PUS1* was sequenced by Sanger/Next-Generation Sequencing. Further clinical investigations and cDNA analysis are ongoing.

Results: In patient 1, compound heterozygosity for NM_025215.5(*PUS1*): c.431G>A p.(Arg144Gln) and c.441 +1G>A was detected. Patient 2 was shown to be homozygous for c.441+1G>A. This mutation is predicted to disrupt the donor splice site of intron 3, making aberrant splicing likely. The missense variant c.431G>T p.(Arg144Gln) is located both at a highly conserved nucleotide and amino acid. Both patients have high S-lactate and chronic acidosis. Ringed sideroblasts were observed only once, in patient 1, but have otherwise not been demonstrable in our patients.

Conclusions: cDNA analysis might give further insights into the consequences of the identified mutations, possibly explaining the long survival. Classical sideroblastic anemia is not obvious in all MLASA patients, and the expressivity and age of presentation of symptoms are variable. Darbepoetin alfa seems effective in controlling the anemia, while severe lactacidosis is difficult to treat.

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P06.37D

Maple syrup urine disease mutation spectrum in a cohort of 40 consanguineous patients; insilico analysis of novel mutations

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Maple syrup urine disease (MSUD) is the primary aminoacidopathyaffecting branched-chain amino acid (BCAA) metabolism. The disease is mainly caused by the deficiency of an enzyme named branched-chained α-keto acid dehydrogenase (BCKD), which consist of four subunits, and encoded by *BCKDHA,BCKDHB, DBT*, and *DLD* gene respectively. Consanguinity increases the chance of the presence of pathogenic mutations in a homoallelic state. Bearing this on our minds, autozygosity mapping was performed with two sets of multiplex polymorphic STR (Short Tandem Repeat) markers linked to the above-mentioned genes. The aim was to indirectly find the probable mutated gene in the studied families. Then families who showed a homozygous haplotype for the STR markers flanking gene/genes were subsequently sequenced. In this study, we also summarized our recent molecular genetics findings to illustrate the mutation spectrum of MSUD in our country with high rate of consanguineous marriage (38.4%). Eleven novel mutations and some recently reported mutations were identified. We found that BCKDHB mutation is the most common mutated gene in our study which is different from other reports in other parts of the world. Additionally, structural modelling of MSUD mutations has been analyzed to predict the pathogenicity of the newly identified variants. Identification of these mutations will further expand the spectrum of known gene mutations and contributes to the genotype-phenotype correlation and is very important in prenatal molecular diagnosis of MSUD at- risk families.

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P06.38A

Identification and characterization of new *RNASEH1* mutations associated with PEO syndrome and multiple mtDNA deletions

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Mitochondrial DNA (mtDNA) depletion and deletion syndrome encompasses a group of disorders caused by mutations in genes involved in mtDNA replication and maintenance. The clinical phenotype ranges from fatal infantile hepatocerebral forms to mild adult onset progressive external ophthalmoplegia (PEO). We report the case of a patient with PEO and multiple mtDNA deletions, with two new homozygous mutations in *RNASEH1*. The first mutation (c.487T>C) is located in the same catalytic domain as the four previously reported mutations, and the second (c.258_260del) is located in the connection domain, where no mutations have been reported. *In silico* study of the mutations predicted only the first mutation as pathogenic, but functional studies showed that both mutations cause loss of ribonuclease H1 activity. mtDNA replication dysfunction was demonstrated in patient fibroblasts, which were unable to recover normal mtDNA copy number after ethidium bromide-induced mtDNA depletion. Our results demonstrate the pathogenicity of two new *RNASEH1* variants found in a patient with PEO syndrome, multiple deletions, and mild mitochondrial myopathy. This work was supported by the Spanish Instituto de Salud Carlos III, Fondo de Investigaciones Sanitarias; EGA is the recipient of a grant from the FIS PI15-01428-FEDER. JT was funded by a fellowship granted by the *Generalitat de Catalunya* (PERIS program, SLT002/16/00370).

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P06.39B

Novel mutation m.13091T>C in *MT-ND5* gene leads to MELAS phenotype - retrospective study in Czech patients with mutations in mtDNA-encoded subunits of complex I

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Isolated respiratory chain complex I (CI) deficiency, the most common cause of mitochondrial disorders (MD), may be caused by mutations in nuclear or mitochondrial DNA (mtDNA). We analysed patients with maternally inherited multisystem MD due to mutations in mtDNA-encoded subunits (MT-ND genes) of complex I diagnosed in our department.

Material: In the cohort of 106 unrelated families with mtDNA mutations, mutations in *MT-ND* genes were found in 13 patients from 12 families.

Results: Among 13 patients, 8 different heteroplasmic mtDNA mutations in *MT-ND1* (2x), *MT-ND3* (1x) and *MT-ND5* (5x) genes were found. In patient with childhood-onset of migraines followed by stroke-like episodes later in adulthood, novel heteroplasmic mutation m.13091T>C in *MT-ND5* was found. Six patients with heteroplasmy levels >60% developed Leigh syndrome and their prognosis was significantly worse compared to five patients with heteroplasmy levels <60%. MELAS syndrome including stroke-like episodes was observed in these five patients. In two children, the diseases started with optic neuropathy mimicking LHON syndrome, later it transitioned to multisystem diseases compatible with MELAS syndrome. CI activities in muscle mitochondria were decreased in most patients and mitochondrial energy-generating capacity was altered in some patients.

Conclusions: Patients with multisystem MD due to mutations in mtDNA-encoded subunits of complex I usually develop Leigh or MELAS syndromes and represent 11% of families with maternally inherited MD diagnosed in our centre. Early onset of the disease and higher mtDNA mutation heteroplasmy levels are accompanied with Leigh phenotype and worse prognosis.

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P06.40C

The mutation spectrum of NAGLU in mucopolysaccharidosis type III B patients

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Aim: Mucopolysaccharidosis type III B (MPS IIIB) is an autosomal recessive lysosomal storage disease caused by

mutations in the *NAGLU* gene which codes the lysosomal enzyme alpha-N-acetylglucosaminidase. The major symptoms of the disease are cognitive and neurological defects. In this study molecular spectrum of 13 MPS IIIB patients were evaluated.

Materyal and Methods: Twelve MPS IIIB patients from 11 families both clinically and molecularly diagnosed were included in this study. *NAGLU* gene sequencing was performed using a next generation sequencing platform (Illumina MiSeq). Demographic, clinical and laboratory findings of the patients were obtained from the hospital records.

Results: Ten different mutations in 13 MPS IIIB patients were identified. Eight of the all mutations were missense, one mutation was splice site and one large deletion. Three mutations (c.509G>T, c.700C>G, and c.1000G>A) were defined for the first time in this study.

Conclucion: Our study expanded the mutation spectrum of the *NAGLU* gene, contributing to the improved genetic counseling of MPSIIIB patients. In accordance with the literature, missense mutations were also the most common mutations in our study.

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P06.41D
Diagnostic exome in neurometabolic disorders

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Introduction: Molecular diagnosis of neurometabolic diseases is challenging due to the large number of established and candidate disease genes associated with an extreme heterogeneity of clinical presentations at variable ages. Especially in infancy-onset of inborn errors of metabolism an early diagnosis is crucial to guide downstream clinical management and treatment decisions.

Materials and Methods: We here report on the results of exome-based diagnostics of **209** index cases with clinically suspected neurometabolic diseases. Coding genomic regions were enriched with a SureSelect Human All Exon Kit V6/7 (Agilent technologies) for subsequent sequencing on an Illumina HiSeq2500/NovaSeq6000 system. Clinical variant prioritization included different filtering steps (e.g. MAF < 0.1 % in gnomAD, in-house database).

Results: In 83 cases (40%), we identified likely pathogenic or pathogenic variants in genes that have been associated with mitochondrial diseases (42), other neurometabolic diseases (21) or other neurodevelopmental disorders (20). In 58 cases (28%) we identified variants of unknown significance where follow up studies for functional analyses are pending. Moreover we newly identified 8 candidate genes affecting the mitochondrial function or selenoprotein metabolism. However 27% of the cases remain unsolved. Further ongoing investigations of the latter in a research setting include the additional analysis of parental DNAs (trio analysis) as well as full genome, transcriptome and proteome analysis.

Conclusions: Although presenting with a wide phenotypic spectrum WES facilitated a definite diagnosis in 40% and a possible diagnosis pending follow up in 28% of the cases.

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P06.42A
The study of the full spectrum of variants leading to hyperphenylalaninemia have revealed 10 new variants in the *PAH* gene

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Introduction: Hyperphenylalaninemia (HPA) is a group of disease characterized by elevated concentrations of phenylalanine in blood. HPA includes phenylketonuria and BH4-deficient forms of hyperphenylalaninemia.

Materials and Methods: 1265 unrelated probands from Russia were previously searched for 25 frequent mutations in the *PAH* gene. DNA samples of 293 probands without or with one frequent mutation were analyzed. The next generation sequencing (NGS) of *PAH*, *PTS*, *GCH1*, *PCBD1*, *QDPR*, *SPR* and *DNAJC12* genes to search for point mutations and MLPA method to search for gross deletions were conducted.

Results: Among 327 chromosomes without identified mutations, mutations in the *PAH* gene were found on 259 chromosomes, and mutations in the *PTS* gene were found on 10 chromosomes. On 10 chromosomes gross deletions by the MLPA method were detected. 104 rare variants of the *PAH* gene, including 10 variants not previously described (p.Pro69Thr, p.Leu83Trpfs*9, p.Gln232Pro, p.Cys237Phe, p.Ala300Asp, p.Gly312Ser, p.Thr328Pro, p.Tyr417*, p.Asp435Val, IVS9-1G>C), and 6 variants of the

PTS gene were revealed. Throught the use all the presented methods, mutations were found on 97.3% of chromosomes.

Conclusions: According to the study results, a spectrum of mutations leading to hyperphenylalaninemia in Russia was established. The data obtained helped to reveal rare mutations of the *PAH* gene and establish the percentage of tetrahydrobiopterin-dependent HPA forms in Russia - 0.4%.

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P06.43B
Correlation of BDNF, LY86, ABCG2 polymorphisms and obesity in an Romanian Caucasian male cohort

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Introduction: Obesity is one of the most important public health problems worldwide. In Romania, the prevalence of obesity is increasing and it is more common in male. The aim of this study is to identify the genes involved in the predisposition and pathogenesis of obesity. 3 allelic variants of genes related to this condition were analysed: rs11030104 of BDNF gene, LY86 gene rs2199936 polymorphism and rs2199936 of ABCG2 gene.

Methods: This study consists of 5281 male patients hospitalized between 2012 and 2017 in multiple clinics in Bucharest for various medical reasons. All subjects were of self-reported European descent. No significant difference was observed between the average age of the cases (66.9) and controls (64.3). DNA was extracted from whole blood at deCODE Genetics (Reykjavik, Iceland) and genotyped, using Infinium OmniExpress-24 bead chips (Illumina). A total of 716 503 SNPs were genotyped for each individual included in the study.

Results: The BDNF rs11030104 polymorphism has shown statistical correlation for obesity alike with the worldwide rates (p=0.0004642). Another statistical correlation between obesity and the rs1294410 LY86 variant, responsible for the waist to hip ratio variation, was identified (p=0.003984). ABCG2 rs2199936, which is involved in the serum uric acid adjustment, has also shown statistical correlation for obesity (p= 0.003733; OR= 0.4707).

Conclusions: The results suggest that in the European male population there is a strong correlation between the BDNF, LY86, ABCG2 polymorphisms and obesity. The research is part of the EUFP7 ProMarka and ROMCAN studies. Replication studies for confirmation of these markes are ongoing.

O. Andrei: None. **P. Iordache:** None. **G. Ursu:** None. **V. Radoi:** None. **L. Bohiltea:** None. **R. Ursu:** None.

P06.45D
Gene discovery for favourable adiposity using cross-phenotype analysis

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Obesity has become a global pandemic in the last few decades. It is often associated with cardiometabolic comorbidities such as cardiovascular disease and type 2 diabetes. However, some individuals remain cardiometabolically healthy despite being obese, also called the metabolically healthy obese (MHO). The biological mechanisms that underlie MHO are poorly understood. To identify novel adiposity-increasing variants with protective cardiometabolic effects, we performed pairwise analyses of adiposity and cardiometabolic traits using available summary statistics from 11 GWAS. These include three adiposity traits (body fat percentage, body mass index, and waist-to-hip ratio) and eight cardiometabolic traits (high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, fasting glucose and insulin, blood pressure, and incident coronary artery disease and type 2 diabetes). We identified genome-wide significant associations ($P<5x10^{-8}$) with any of the 24 trait pairs consisting of one of three adiposity traits and one of the eight cardiometabolic traits. This resulted in the discovery of 62 independent loci residing in gene regions such as *COBLL1, PPARG, VEGFB* and *VEGFA*. Results of tissue and cell enrichment analyses using DEPICT implicate strongest enrichment in adipose tissue, adrenal cortex, liver, and arteries. Gene set enrichment analyses using DEPICT highlighted several pathways ($P<5x10^{-4}$), such as "abnormal muscle cell glucose uptake", "decreased percent body fat", and "response to insulin stimulus". Our results provide insights into biological pathways that may underlie MHO and could shed light on future drug development targeting improvement in cardiometabolic profile of unhealthy obese individuals.

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P06.46A

A clinical case of somatic mosaicism in an unaffected father of female patient with ornithine transcarbamylase deficiency (OTC)

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Introduction: Ornithine transcarbamylase deficiency (OTC) is an X-linked inborn error of metabolism of the urea cycle. OTC deficiency can occur as a severe neonatal-onset disease in males and as a post-neonatal-onset (partial deficiency) disease in females.

Materials and Methods: We present family with 4-years old girl who has clinical symptoms of urea cycle disorders. Another members of family are healthy. Proband's perinatal period was normal. Since the first year she refused to eat meat product. At age of 3 years she was detected liver transaminase elevated (more than 10 norm) with neurological symptoms. Orotic acid concentration in urine was normal. A low-protein diet was effective: a liver transaminase normalized, neurological symptoms stopped.

Results: Analysis of whole exome sequencing data revealed a pathogenic heterozygous variant c.78-1G>A in OTC gene in the proband. This variant was confirmed by Sanger sequencing in proband, wasn't detected in her mother and her healthy older sister, and was detected in her father in heterozygous state in mosaic form. In her father we detected only one copy of X -chromosome by different methods, including cytogenetic. We detected mosaicism of variant c.78-1G>A in OTC gene in the different tissues (buccal epithelium, blood, seminal fluid, urinary sediment) which are having an origin from every germ layer (ectoderm, mesoderm, endoderm).

Conclusions: Our clinical case shows that affected females may inherit the OTC pathogenic variant from her father who doesn't have clinical symptoms of disease. It is important for determination of genetic risk and discussion of the necessary of prenatal testing.

N. Semenova: None. **I. Anisimova:** None. **O. Shchagina:** None. **A. Chuchrova:** None. **O. Ryzhkova:** None. **I. Bychkov:** None. **N. Pechatnikova:** None.

P06.47B

Enrichment of variants affecting mitochondrial metabolism in patients with Parkinson disease

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While age-related accumulation of somatic mitochondrial mutations has been demonstrated in Parkinson disease (PD) patients, the role of germline mitochondrial variants has never been systematically studied. In the present study, we aimed to investigate the germline genetic burden in genes affecting mitochondrial function and the role of the subsequent genetic variability in PD. For this reason, we compared the genetic variability in 70 patients with early onset or familial PD without a discovered monogenic cause with that of 253 controls. Mitochondrial genome was reconstructed from clinical or whole exome sequencing data, which allowed us to investigate both mitochondrial as well as nuclear genes, including nuclear genes involved in human mitochondrial disorders. For further analysis, variants were filtered excluding nuclear variants based on in-house populational data, those with mean allele frequency over 5% in public databases and synonymous mitochondrial variants. We detected significant differences in burden of variants in three mitochondrially encoded genes. The greatest increase of variant burden between PD and controls was observed in MTND5 (38.6% versus 26.9%), MTCO2 (10.5% versus 5.5%) and MTND4L (7% versus 0.5%), with the first and last being mitochondrial respiratory complex I components. No significant differences have been observed in the variant burden of nuclear genes. In conclusion, our findings support the hypothesis that germline mitochondrial variants might contribute to the pathogenesis of PD.

G. Bergant: None. **A. Maver:** None. **B. Peterlin:** None.

P06.48C

Rapid mitochondrial genome (mtDNA) sequencing: facilitating rapid diagnosis of mitochondrial diseases in paediatric acute care

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Introduction: Standard rapid genomic testing techniques analyse nuclear DNA variants using exome and/or genome sequencing (ES/GS). Rapid mtDNA analysis is not routinely available, particularly in centres performing ES, which does not deliver clinical-grade mtDNA sequencing. We describe our experience using rapid mtDNA sequencing in tandem with an ES-based rapid genomic diagnosis program as part of the Australian Genomics Acute Care flagship.

Methods: Two infants presenting with persistent lactic acidosis and bone marrow failure were recruited for rapid genomic testing. With clinical suspicion of mitochondrial disease, both infants underwent rapid ES and mtDNA sequencing in tandem, the latter using Nextera libraries from a full length mtDNA amplicon.

Results: ES was non-diagnostic in both infants. mtDNA sequencing identified a single large mtDNA deletion in both infants, diagnostic of Pearson syndrome (MIM 557000). Diagnostic reports were issued within 73 hours 55 minutes and 54 hours 25 minutes, respectively. Both infants avoided invasive bone marrow biopsies and a range of other investigations.

Conclusions: Rapid mtDNA sequencing in tandem with ES results in additional diagnoses in seriously ill children with suspected mitochondrial pathology, suggesting that ES alone may be insufficient in this setting. When designing rapid genomic diagnosis programs, centres should consider incorporating mtDNA amplification and analysis in individuals with suspected mitochondrial pathology, by combining ES and mtDNA sequencing in tandem, or analysing mtDNA data from GS, which captures the mitochondrial genome.

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P06.49D

Biochemical and molecular genetic diagnosis of Moldavian PKU patients: A novel missense mutation in PAH gene

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Phenylketonuria(PKU) is an inborn error of metabolism involving impaired metabolism of phenylalanine that leads to severe mental retardation. In Moldova PKU diagnosis is based on newborn screening(NBS) from 1989.

Materials and Methods: Diagnosis of 123 Moldavian PKU/HPA patients was based on *Phe* level >3mg/dL and genetic analysis by PCR/RFLP method of hot-spot mutations (*R408W, P281L, R158Q, R261Q, R252W, IVS12 +1G>A, L48S, R261X, G272X, IVS10-11G>A)* carried out on Eppendorf MasterCyclerPro followed by "Sanger" DNA sequencing on ABI 3130xl Genetic Analyzer(Switzerland), ABI 3500dx Genetic Analyzer(Moldova).

The results: During 28 years, 107 PKU patients have been identified by NBS and 16 patients - extra screening, showing the total PKU incidence of 1:6463 newborns, screening rate 92-97% for last 5 years. Most of them(69%) have a classical PKU phenotype(*Phe* levels at diagnosis 24,3-52mg/dl), followed by moderate PKU(21%), mild PKU(7%), and HPA(2%). The 105 PKU patients were subjected to genetic analysis of *PAH* gene being identified 32 disease causing mutations: 18 missense(56%), 8 splicing (25%), 3 deletion(9%) and 3 nonsense(9%) mutations, that compound 47 different genotypes in Moldavian PKU patients. The most common mutation was *R408W* found in 50.5% of cases. One novel mutation *c.2T>C* in exon 1 was identified in a patient with constantly *Phe* level ~13mg/dL, slight intellectual disability and no other mutations in PAH gene was detected, that was suggestive for errors in BH4 biosynthesis genes.

Conclusion: Efficiency of PKU diagnosis in Moldova has been increased, due to NBS coverage and mutation detection over 90% rate, applying a targeted specialized treatment and prenatal diagnosis.

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P06.50A

Whole genome sequencing characterizes precise break points and extent of heterozygous deletion in *PAH* in a patient identified by newborn screening

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Since 1965, newborn babies in Sweden are screened for Inborn Errors of Metabolism (IEM) by biochemical analysis. For the past few years, findings are genetically characterized in our laboratory by exon panel sequencing and Sanger. Here we complement exon panel sequencing with whole genome sequencing (WGS), which is superior

characterizing copy number variations. Exon panel sequencing of a sample positive for phenylketonuria (PKU) on newborn screening detected a heterozygous SNV (single nucleotide variant) in a well known CpG hotspot for PKU, in *PAH*. Copy number analysis (CNV) indicated a heterozygous deletion spanning exon 3 *PAH*, but not exon 2 nor 4. Using whole genome sequencing, we were able to reveal the exact break points and size of the deletion. Exon 3 of *PAH* is involved in encoding the regulatory domain (located at the N-terminal) of phenylalanine hydroxylase. This domain determines the specificity of the enzyme for the amino acid phenylalanine. The break points as well as the SNV, were subsequently verified by Sanger sequencing and both parents were also analyzed by Sanger sequencing to confirm their heterozygous genotypes. The proband was consequently found to be compound heterozygote, resulting in classical PKU.

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P06.51B
Proteostasis regulators as a possible mutation-specific therapeutic approach for PMM2-CDG

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Introduction: PMM2-CDG, the most common glycosylation disorder for which there is no cure, has been suggested to be a conformational disease. The misfolded nature of most of the disease-causing mutations makes the use of stabilizing molecules, pharmacological chaperones (PCs) or proteostasis regulators (PRs), a promising therapeutic strategy. The aim of this study was to evaluate the effect of PRs on the stability and activity of PMM2 unstable mutants and decipher their molecular mechanisms.

Materials and Methods: Patient-derived fibroblasts transduced with their own PMM2 folding or oligomerization mutation were treated with different concentrations of PRs (celastrol and MG-132) or combining celastrol and a PC (CVIII). Their effect was studied by western blot and PMM enzymatic assay. The expression levels of several molecular chaperones (HSPs) were assessed by qRT-PCR and western blot. The specificity of the molecular chaperones involved was evaluated by using specific HSP70 or HSP90 inhibitors.

Results: Celastrol treatment significantly increases PMM2 protein levels and activity of various mutants in the cellular model. These results correlate with an increment in the transcriptional and proteomic levels of some HSPs of the proteostasis network. The HSP90 inhibitor, but not the HSP70 one, interferes with the effect of celastrol on PMM2. The combination of celastrol and CVIII has a mutation-dependent synergistic effect on PMM2 activity.

Conclusions: These results show the positive effect of celastrol on the PMM2 stability and activity through the HSP90-driven modulation of the proteostasis network. This is a proof-of-concept of the possible therapeutic use of PRs and PCs for PMM2-CDG.

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P06.52C
Functional consequences of bi-allelic *PNPT1* variants associated with multisystemic disease

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Introduction: *PNPT1* encodes the Polyribonucleotide nucleotidyltransferase 1 protein which predominantly

localizes to the mitochondria, and is involved in multiple RNA processing functions. Bi-allelic pathogenic variants in *PNPT1* have been identified in patients with a heterogeneous phenotype ranging from non-syndromic deafness to a multisystemic presentation with severe global developmental delay, seizures, dystonia, choreoathetosis, ophthalmological abnormalities and different MRI-findings.

Materials and Methods: We investigated 5 unrelated patients with bi-allelic variants in *PNPT1* identified through massively parallel sequencing. The patients presented with clinical features in common with previously reported multisystemic cases. Using patient fibroblasts and peripheral blood samples, we performed functional studies to confirm pathogenicity including cDNA studies, complex I (CI) and IV (CIV) enzyme activity assays, immunoblotting, RT-qPCR analysis for detection of mitochondrial unprocessed transcripts, and expression of targeted interferon signalling genes.

Results: Compared to controls, patient fibroblasts showed mildly reduced CI and CIV protein activities, there was also a reduction in PNPT1 protein expression and a 2-6 fold accumulation of *MT-ND5+MT-ND6* and *MT-CYTB+MT-ND6* mitochondrial RNA unprocessed transcripts. In blood, there was an increased expression of 6 genes involved in the interferon I pathway.

Conclusions: The accumulation of mitochondrial unprocessed transcripts and increased interferon pathway expression provides further evidence for the role of PNPT1 in mitochondrial RNA processing, and of the possible contribution of an immune response to disease pathogenesis. It also demonstrates that in order to confirm pathogenicity, functional analyses of the primarily affected processes are more sensitive than the modest defects detected downstream on the respiratory chain enzyme activities.

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P06.53D

Whole exome sequencing (WES) in suspected mitochodrial disease: *PUS1* gene and MLASA spectrum

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Mitochondrial diseases (MD) are the most common cause of inherited metabolic diseases. Combined respiratory chain (RC) complexes defects are caused by mutations in the mtDNA-encoded RNA products and mutations in nuclear genes involved in mtDNA translation machinery. *PUS1*, a nuclear gene, encodes for cytoplasmatic and mitochondrial tRNA pseudouridine-synthase-1, that converts uridine into pseudouridine in several tRNA positions increasing the efficiency of protein synthesis on both compartments. Mutations in *PUS1* cause MLASA syndrome, a rare autosomal recessive disorder of oxidative phosphorylation and iron metabolism, clinically characterized by Mitochondrial Myopathy, Lactic Acidosis and Sideroblastic Anemia. We report clinical, biochemical and molecular findings of unreported patient in which Whole Exome Sequencing (WES) revealed the presence of new biallelic mutations in *PUS1*. Review of the 16 *PUS1* mutant patients published to date demonstrates a huge clinical heterogeneity with intellectual disability, microcephaly and anemia as typical clinical elements. Given the paucity of the patients described, it is not possible to delineate a complete phenotypic picture and the associated clinical evolution, and it is not possible to establish an accurate genotype - phenotype correlation that allows us to indicate a proper prognosis and follow-up. Moreover, our patient had severe osteoporosis with multiple fractures that were never previously reported in the literature and a more early evolution of neuro-muscular symptoms than the other patients. WES is increasing the diagnostic yield in MD, in particular enhancing the ability to identify potential nuclear gene mutations in patients with biochemically defined defects affecting multiple mitochondrial RC and complex phenotype.

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P06.54A

Mitochondrial disease caused by a novel homozygous mutation (Gly106del) in the SCO1 gene

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Introduction: The Cytochrome C oxidase assembly protein *SCO1* gene encodes a mitochondrial protein essential for the mammalian energy metabolism. Only three cases of *SCO1* mutations have thus far been reported. All cases presented with lactate acidosis and encephalopathy. Two had hepatopathy and hypotonia. One further presented with intrauterine growth retardation and hypertrophic cardiomyopathy leading to cardiac failure.

Here we present a case of a girl of Afghan consanguineous parents, born premature with a novel homozygous mutation in the *SCO1* gene and a different phenotype than previously reported cases.

Within the first two hours, the girl developed severe lactate acidosis and hypoglycemia. Echocardiography, eye examination, ultrasound of the brain, kidneys and urinary tract were normal. As was the level of ammonium. At four days old an investigation of suspected metabolic disease was initiated including whole exome sequencing. Merely five days later whole exome sequencing revealed a novel homozygous mutation in the *SCO1* gene (c.317_319delGAG) in exon 2 resulting in an in-frame deletion of Gly106. A muscle biopsy was later performed, showing reduced activity of complex IV in accordance with the *SCO1* mutation detected by whole exome sequencing. The girl died 1 month old.

Conclusion: We identified a novel homozygous *SCO1* mutation (Gly106del) in a patient with a clinical course of mitochondrial disease different from previously reported *SCO1* cases. Mitochondrial disease may manifest in neonates, but early diagnosis has so far been difficult. Because of improved technique as whole exome sequencing early diagnosis and consideration of treatment level are now possible.

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P06.55B
Characterization of seven mitochondrial DNA variants of unknown significance by quantification of single cell heteroplasmy

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Mitochondrial dysfunction is associated with a variety of diseases. Each human cell contains several mitochondria which hold multiple copies of mitochondrial DNA (mtDNA). mtDNA heteroplasmy is the coexisting of wild-type and mutant mtDNA molecules in a cell. The proportion of mutated mtDNA should exceed a threshold to cause biochemical defects. NGS leaded us to identify an increasing number of pathogenic variants but also of variant of unknown significance (VUS).Only a tiny sub-group of VUS was functionally evaluated with certitude, while the pathogenicity of the great majority was only assessed in-silico. We investigated seven patients with clinical features, muscle biopsy and biochemical defects suggesting a mitochondrial dysfunction, including the presence of cytochrome c oxidase - deficient fibers (COX (-)). mtDNA sequencing found a VUS for each patient. In order to determine the pathogenicity of these VUS, we quantified the level of mtDNA heteroplasmy by semi-quantitative fluorescent PCR in muscle fibres COX (+) and COX (-) single cells. We found a statistically significant heteroplasmic difference between individual COX (+) and COX (-) cells for 6 patients, which supports the pathogenicity of these VUS, allowing us to confirm the diagnosis for these patients. Determining the pathogenicity of the VUS identified by NGS is becoming a major goal to reduce diagnostic wandering in patients. The single cell heteroplasmy quantification is a gold-standard method for the mutation-disease causal link assessment and should be used routinely in diagnosis. In this objective, we designed a fastest and easiest single cell quantification tool.

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P06.56C
TANGO2 associated autosomal recessive disease: A novel case

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Introduction: TANGO2-related disorder was first published in 2016. Homozygous or compound heterozygous mutations of TANGO2 gene lead to the disease Metabolic encephalomyopathic crises, recurrent, with rhabdomyolysis, cardiac arrhythmias, and neurodegeneration. Symptoms include recurrent metabolic crises associated with encephalopathy, rhabdomyolysis, cardiac arrhythmias and

laboratory findings such as hypoglycaemia, hyperammonemia, lactic acidosis and elevated CK levels. With the progression of the disease epilepsy, developmental delay, cognitive impairment, global brain atrophy and pyramidal signs appear.

Materials and Methods: We report a 4-year-old child with developmental delay. From 21 months of age he showed episodes of suddenly onset hypotonia, gait instability, ataxia, and distortion of the head and upper body. During these paroxysm plasma creatinine kinase and lactate was elevated. He has seizures from 2-years-of-age: grand mal and spasms also occurred. He has hypothyreoidism.

Results: A pathogenic apparently homozygous TANGO2:c.385C>T variant was identified in exon 4 of the TANGO2 gene by Whole Exome Sequencing. We examined the parents for the above mentioned variant, but we could only detect the variant in the mother. Based on that further examinations were needed to distinguish in between homozygosity versus heterozygosity with a deletion. With quantitative PCR assay by using 3 gene-specific amplicons encompassing the coding exons 3,4,5 of the TANGO2 gene a large deletion encompassing exons 3-5 was detected.

Conclusions: The above mentioned compound heterozygous state in our patient confirms the diagnosis of TANGO2-associated biallelic disorder. The disease might be very underdiagnosed, whole exome sequencing is still a clue to find the alterations in TANGO2 gene.

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P06.57D

Adult form of Tay-Sachs disease: main symptoms, enzyme and genetic diagnostics in Czech patients

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Tay-Sachs disease (TSD) is a rare neurodegenerative disorder caused by lysosomal β-hexosaminidase A deficiency due to mutations in the *HEXA* gene. Phenotypic spectrum of TSD is diverse and differs between infantile and adult form. No effective treatment for TSD is available and genetic counselling (DNA analysis) is thus important for prenatal diagnosis. Our cohort includes 14 patients with adult form of TSD (surprisingly twice more than infantile form) diagnosed in the Czech Republic between 2002 and 2018 yielding an estimated birth prevalence of 1:325,000. Median age of first symptoms and of diagnosis were 21 years and 35 years, respectively. The main clinical symptoms were problems with mild dysarthria, rapid speech, proximal weakness of lower extremities, balance disturbances and/or psychiatric disorders. Cerebellar atrophy in brain MRI was a constant feature. Leukocyte hexosaminidaseA activity was decreased to 1.8 - 4.1% of controls. All patients carried at least one allele with the mild variant p.Gly269Ser; a novel variant p.Arg252Cys was detected in one family. Adult form of TSD seems to be an underdiagnosed although an enzyme assay in serum/plasma is a fast, inexpensive and reliable tool to detect patients with this form of TSD and genetic confirmation is routine.

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P06.58A

TRMU-related transient liver failure of infancy presents with microcephaly and neurodevelopmental delay

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21 months-old girl presented at genetics outpatient clinics due to microcephaly, mild neurodevelopmental delay and facial dysmorphism. She was born to healthy non-consanguineous couple. Prenatal and perinatal history was uneventful. She was followed up at metabolism& gastroenterology clinics for vomiting attacks, elevated liver enzymes/ lactate levels during 3-to-12 month-of-age. Mitochondrial work-up had not revealed the underlying pathology. SNParray showed 16q23.1 deletion encompassing two genes, *WWOX* and *LSM3P5*, which is associated with bilateral cataracts, iris coloboma and autistic behaviour. Paternal transmission of the microdeletion was conclusive for its possibly benign nature. Clinical Exome Sequencing(CES) revealed homozygous *TRMU gene* c. G835A(c.V279M) pathogenic change explanatory for the history of transient liver failure of infancy. FibroScan at $2^{1/2}$ years revealed stage2 hepatosteatosis with mild fibrosis and liver biopsy was performed for histopathological evaluation. *TRMU* (tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase) gene mutations were identified as responsible from transient liver failure of infancy at 2009.

Twenty-two patients have been reported uptill now and clinical variability ranges from early death(6/22) to normal development. Although no systematic follow-up is available, microcephaly and mild neurodevelopmental delay has been reported. The case we reported herein presented with microcephaly and psychomotor retardation and diagnostic work-up revealed the etiopathogenesis of transient liver failure in infancy. CES as the leading diagnostic technology for single gene disorders, enables clinical management, counselling also for TRMU-related hepatic failure which has a relatively benign course. *TRMU* gene sequencing is highly recommended in infants with unexplained liver failure not only in Yemenite Jewish but in all Middle East populations and Turkey.

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P06.59B
Genetics meets Metabolomics in Type 2 Diabetes in a Middle Eastern Population

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T2D and its complications are a major concern for its high prevalence in Qataris, and both genetic risk and lifestyle/environmental factors play a role in T2D. We investigate: Polygenic Risk Scores (PRS) of T2D in Qataris, genetically Determined Metabotypes (GDM) of T2D and gene-metabolic networks. Polygenic risk scores highlight the role of genetics in early diagnosis, compared to other risk factors. Identifying gene-metabolite associations is important for understanding the genetic factors affecting metabolic pathways. 1000 Qatari samples (57% T2D), with whole exome sequence and genotype array data were used. All samples were profiled for more than 1000 metabolites. We constructed a PRS using those SNPs that replicated from 81 published loci, in the array data, and computed the accuracy of PRS in individuals with whole exome data. For determining GDMs in T2D, we first identified metabolites significantly associated with T2D, and then the associations of those metabolites with 1.6 million common variants. We found 229 metabolites associated with T2D, among which 6 metabolites are significantly associated with SNPs in 5 loci. We identified a gene-metabolic network containing 21 unique loci and 33 unique metabolic pathways. In this study we show genes that contribute to the polygenic risk score of T2D, genetically determined metabotypes and gene-metabolic networks in T2D. We believe these results will help improve the understanding of T2D mechanisms and in early diagnosis of the disease.Acknowledgement: This work was supported by Biomedical Research Program in WCM-Q, and by Qatar National Research Funds grants numbers: 09-740-3-192 and 09-741-3-793.

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P06.60C
High-throughput metabolomics for early detection of individuals at increased risk for type 2 diabetes

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Introduction: Advances in metabolomics now allows for profiling of large-scale cohorts. We identified circulating blood biomarkers predictive of T2D. Also, metabolic signatures were generated for risk stratification and tracking the health of the individuals.

Materials and Methods: NMR metabolomics was used to quantify 228 metabolic measures in 11,896 individuals from four Finnish cohorts (mean age 35 years, range 24-45).

Results: Associations between baseline metabolites and diabetes onset during the 7-15 years of follow-up (392 incident cases) were assessed by logistic regression. Altogether 113 metabolites were associated with incident diabetes ($P<0.0009$; range of odds ratios (OR) per 1-SD: 0.59-1.50) after adjusting for sex, baseline age, glucose and BMI. Among the strongest predictors of increased risk of diabetes were higher concentrations of branched-chained and aromatic amino acids (OR: 1.33), triglycerides in very-low-density lipoproteins (VLDL; OR 1.50), and lower levels of omega-6 fatty acids (OR 0.75). A biomarker signature comprised of phenylalanine, free cholesterol in large HDL, and the ratio of cholesteryl esters to total lipids in large VLDL was predictive of incident diabetes in an independent validation cohort after adjusting for baseline glucose and BMI (OR 10.1 comparing 5th vs 1st quintile of the biomarker score).

Conclusions: Individuals at risk for T2D display a distinct metabolic signature even years before the disease develops. Using the signature, both early risk stratification and personalised follow-up of the disease progression is enabled. High-throughput metabolomics therefore provides a powerful tool for population-wide diabetes prevention and control programs. Metabolomics is already used for health tracking in Finland.

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P06.61D
From prenatal cystic hygroma to rare metabolic disease-a patient with Zellweger Syndrome

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Introduction: Zellweger syndrome (ZS) is a rare, fatal, autosomal, recessive inheritable disease that occurs as a consequence of mutations in one of the 14 PEX familial genes. ZS represents the absence of peroxisomes resulting in damaging metabolic pathways, especially beta oxidation of very long chain fatty acids. ZS is characterized by facial dysmorphia, neurological disorders and gradual insufficiency of all vital organs. In the second half of pregnancy the ventriculomegaly appearance is possible and endocranium MRI could detect characteristical ZS changes: abnormal gyral forms, disturbed myelination and cerebral periventricular pseudocysts.

The aim is to use the case report on a ZS patient to point out the possibility of recognising a rare inheritable disease expected with a frequency of one in 50,000 live births.

Results: The infant with dysmorphia, hypotonia, hyperbilirubinaemia, and hepatomegaly is presented. In 13th week of gestation the enlarged nuchal region cystic hygroma and the border ventriculomegaly were found by ultrasound examination. Pre-natal diagnosis excluded chromosomal aberrations and pregnancy was continued and completed by birth in term. Severe hypotonia and dysmorphia were present at birth. In the further course a jaundice, rise in liver enzymes and lactate dehydrogenase, development of hepatic insufficiency, and multiorganic failure have occurred with a lethal outcome in the fifth month of life. A clinical exome sequencing (NGS) identified the homozygous mutation in the PEX6 gene, described as pathogenic-causative to ZS.

Conclusion: Considering the characteristics of ZS, setting up a suspicion and conducting prenatal diagnosing for ZS are crucial in preventing this syndrome among liveborns.

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P07
Immunology and hematopoietic system

P07.01A
Shared immunogenetic associations across common pathogens discovered through Multiplex Serology screening in UK Biobank

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Introduction: Infectious agents contribute significantly to the global burden of disease, through both acute syndromes and chronic disease sequelae. Understanding the biological mechanisms underpinning susceptibility to infection and development of chronic disease may inform future preventative and therapeutic strategies.

Materials and Methods: Using a rigorously validated Multiplex Serology platform, we sought to identify human genetic variation associated with differential response to infectious agents. We undertook genetic association analysis of antibody responses measured against 45 antigens targeting 20 infectious agents implicated in chronic disease pathogenesis (including human herpes and papillomaviruses, and *Helicobacter pylori*) using baseline serum samples from 9,611 individuals selected at random from the UK Biobank.

Results: We identified 2853 non-HLA regions associated with antibody responses to our selected pathogens ($p < 1x10^{-5}$), 13 of which contained peaks reaching genome-wide significance ($p < 5x10^{-8}$). Some of these association signals validate candidate mechanisms of infection activity whereas others implicate other plausible yet previously unrecognised molecular pathways. Associations were observed within the extended MHC region for at least one antigen from each of the 20 pathogens. Of the non-MHC

associations, we identified three regions associated with more than one pathogen. These regions span *NFKB1* on chromosome 4, the *IGH* region on chromosome 14, and *FUT2* on chromosome 19.

Conclusions: The findings and approaches used in this work and future ambitions to apply the Multiplex Serology platform to the entire UK Biobank cohort are likely to improve our understanding of infection-disease associations and molecular mechanisms facilitating therapeutic discovery and precision medicine.

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P07.03C

Study of *TNF, IL1B*, and *IL6* genes polymorphisms and susceptibility to bronchopulmonary dysplasia in premature neonates

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Introduction: Bronchopulmonary dysplasia (BPD) is a common chronic lung disease associated with preterm birth. Our objective was to investigate whether the *TNF, IL1B*, *IL6* genes polymorphisms have influence on BPD susceptibility.

Materials and Methods: The study included 351 neonates with gestation period less than 32 weeks. Genotyping of *IL1B -511 G/A*, *IL6 -174 G/C* and *TNF -308 G/A* variants was performed by Real-time PCR. The statistical differences in genotypes distribution among the groups were compared using the chi-square or Fisher exact test.

Results: BPD was present in 36 neonates (11.43%). There were no significant differences in the genotypes distribution and allele frequencies for the *IL1B* and *IL6* genes variants between neonates with and without BPD. The frequencies of *TNF* genotypes were GG 55.6%, GA 38.9% and AA 5.6% in neonates with BPD and GG 79.7%, GA 19.7% and AA 0.6% in neonates without BPD. *TNF* genotypes frequencies between these groups were statistically significantly different (p=0.001). Also, statistically significant difference was observed in *TNF* genotypes distribution between neonates with BPD and without BPD by dominant model (GG vs. GA/AA) (p=0.001; RR=2.188, 95% CI 1.428-3.348; OR=3.138, 95% CI 1.539-6.397). After the adjustment for BMW, gestational age, and sepsis, logistic regression confirmed statistically significant association between *TNF -308G/A* genotypes and development of BPD in premature infants (B=- 0.992, p=0.003). *TNF -308 A* allele is risk factor for BPD (p=0.0007).

Conclusions: Our findings suggest that *TNF -308 A* allele is a risk factor for development of BPD in premature neonates under the 32 weeks.

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P07.04D

Hereditary angioedema due to C1-inhibitor deficiency in south-eastern Europe: *SERPING1* mutations and genetic factors modifying the clinical phenotype

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Introduction: Hereditary angioedema due to C1 inhibitor deficiency (C1-INH-HAE) is a rare genetic disorder characterized by recurrent oedemas and large heterogeneity in clinical presentation. Our aim was to determine the spectrum of *SERPING1* mutations in C1-INH-HAE patients, including genotype-phenotype relationship and if functional genetic variants in *F12* and *KLKB1* affect the disease expression.

Materials and Methods: A cohort of 150 clinically well characterised C1-INH-HAE patients from 75 unrelated families from Croatia, Republic of Macedonia, Serbia, and Slovenia was recruited for genetic analysis, which included sequencing and MLPA analysis of *SERPING1*, as well as detection of *F12* (rs1801020) and *KLKB1* (rs3733402) variants.

Results: We have identified 39 different mutations (14 missense, 11 nonsense, 7 frameshift, 1 in-frame deletion, 2 splicing defects, 1 substitution affecting the promoter, 3 large indels). Thirteen mutations have not been previously

described. When addressing the genotype-phenotype relationship we found that patients with nonsense and frameshift mutations, large indels, splicing defects, and mutations affecting the C1-INH active site (at Arg444) exhibited an increased clinical severity score, compared with those with missense mutations, excluding mutations at Arg444. Importantly, the *F12* variant was associated with disease onset. No association between *KLKB1* variant and disease expression was identified.

Conclusions: Our study identified 39 different, among them 13 novel, disease-causing mutations in C1-INH-HAE patients, highlighting the heterogeneity of mutations in the *SERPING1* gene. The mutations with a clear effect on C1-INH function predispose patients to a more severe disease phenotype and the CC *F12* variant to earlier disease onset.

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P07.05A
Functional study of the rs6822844 SNP associated with risk to celiac disease in intestinal cells

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SNP rs6822844 shows the strongest association with celiac disease (CeD) susceptibility outside the HLA region. The SNP is located between IL2 and IL21 genes, which encode for immune IL-2 and IL-21 cytokines, respectively, and have been proposed as candidate etiological genes for CeD, although functionally confirmation is still pending. Thus, the main aim of this study was to analyze the effect of rs6822844 in intestinal cells. For that purpose, the genomic region surrounding rs6822844 was mutated in HCT116 epithelial intestinal cells using CRISPR-Cas9, and mRNA levels of IL2, IL21 and FGF2 were analyzed. The CRISPR-Cas9 edition produced a complex reorganization of the genomic region, and provoked a downregulation of FGF2 expression located 240kb downstream of the SNP. On the contrary, FGF2 expression was upregulated in intestinal biopsies from CeD patients. In addition, U937 monocytes were incubated in edited and non-edited HCT116-conditioned media and ILB1 expression was measured to evaluate their activation. Proliferation assays were also performed in both HCT116 and U937 cells. Monocytes incubated in conditioned medium from edited HCT116 cells showed lower activation and slower proliferation than those incubated in medium conditioned by wild-type HTC116 cells. Unlike, edited HCT116 cells presented a higher proliferation rate compared to wild type cells. In conclusion, disruption of the rs6822844 region provokes alterations in intestinal cells that seem to affect monocytes and in turn the inflammatory status, suggesting the presence of genomic elements within the region that could contribute to the development of celiac disease. Funding: PI16/00258 (JRB) and EJ-2017111082 and ACM (ACR).

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P07.06B
Immunoregulatory effect and the role of plasma cell-free DNA in celiac disease

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Introduction: Immunoregulatory effects of plasma cell-free DNA (cfDNA) have been reported but its role in pathogenesis of celiac disease (CD) has not been studied yet.

Materials and Methods: We determined the total cfDNA concentration and relative content of telomeric sequences in plasma cfDNA in recent celiac disease patients (rCD, n = 10) and healthy age-matched controls (HC, n = 10) by qPCR. To document that the observed biological effects are caused by cfDNA molecules, we treated the paired plasma samples with DNase. Using paired samples of plasma (non-treated and treated by DNase), we analyzed the contribution of cfDNA to the activation of TLR9 and TNF-α mRNA expression in THP1 monocytic cell line.

Results: We found neither significant differences in the quantity of cfDNA nor in the relative amount of telomere sequences in the non-treated rCD and HC plasma. Stimulation of THP1 cells with non-treated rCD plasma led to higher mRNA expression level TNF-α ($p=0.031$) than stimulation with non-treated HC plasma. The cfDNA in rCD plasma samples stimulated the production of TLR9 mRNA.

The TLR9 mRNA expression was significantly ($p = 0.014$) lowered after cfDNA removal from rCD plasmas.

Conclusions: We provide the first evidence that cfDNA contained in rCD plasma differs in its immunoregulatory capacity from cfDNA in HC plasma.

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P07.07C
Machine learning classifiers implicate B cell activation in chronic fatigue syndrome

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Chronic Fatigue Syndrome (CFS) is a disease resulting in extreme fatigue without any known underlying medical condition. CFS often presents in patients following a viral infection and patients may appear to have slightly impaired immune systems. With no positive diagnostic, CFS is often misunderstood and misdiagnosed. A pilot clinical study was established to give further insight into the immunological role of CFS. This pilot study included 15 CFS patients and 15 age and sex matched controls undergoing cardiopulmonary exercise testing (CPET). Blood was extracted at four time points - immediately before, 1 day after, 2 days after and 3 days after. Five immune cell types were sorted from whole blood collected from each participant, and RNA was extracted and sequenced for each cell type, as well as whole blood, at each time point. Additionally, each participant underwent extensive clinical surveys and questionnaires.

Our team has employed machine learning approaches to construct classifiers that can accurately distinguish CFS from non-disease states in immune cells using RNA-Seq data. This technique has generated non-linear gene associations that can be used to understand distinct gene expression differences between disease and non-disease states. We were able to identify B cell classifiers as being the highest performing classifiers in the immune cell populations, suggesting B cells contain more interesting disease-state biology. Gene signatures from these classifiers were further found to be enriched in co-expression modules associated with B cell maturation. These classifier algorithms can also be used to predict unseen observations as a future diagnostic tool.

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P07.08D
Angiogenesis in CML: miRNAs and angiogenic factors in plasma before and after TKI treatment

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Introduction: The differences of an angiogenic potential between clinical phases of chronic myeloid leukemia (CML), point at the significance of neovascularisation in CML pathogenesis. Tyrosine kinase inhibitors (TKI), possessing antiangiogenic properties and microRNAs (miRNAs), implicated in governing angiogenesis, both seem vital in this process. In this study, we aimed to investigate how TKI treatment affects angiogenesis-related miRNAs expression and angiogenic factors concentration in plasma of CML patients.

Materials and Methods: Peripheral blood plasma samples were obtained from CML patients at the diagnosis (n=23) and during TKI treatment (n=12). Quantitative assessment of the expression of miRNA-126-3p, miRNA-150-5p and miRNA-21-3p was performed with qRT-PCR. Concentrations of selected angiogenic factors in plasma (Angiogenin, bFGF, Endostatin,aFGF, PDGF-AA, PIGF, Thrombospondin-2, VEGF-D, Angiopoietin-1, VEGF) were assessed using multiplex fluorescent bead-based immunoassays (Luminex Corporation).

Results: MiRNA-150-5p and miRNA-21-3p expression was evidently higher in the treatment group (p=0,001 and p=0,03, respectively). PDGF-AA concentration in newly diagnosed patients with CML was higher than in already treated patients (942,84 pg/ml vs 512,5 pg/ml, p=0,02), similarly to VEGF (262,82 pg/ml vs 27 pg/ml, p=0,03).

Conclusions: TKI treatment affects miRNAs expression and angiogenic factors concentration in plasma of CML patients. MiRNAs could serve as biomarkers in monitoring CML progression and drug response, however, their exact role in CML-related angiogenesis remains to be further elucidated.

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P07.09A

Increasing fetal hemoglobin by genetic editing the cells of sickle cell disease patients

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Beta-hemoglobinopathies, such as Sickle Cell Disease (SCD) and beta-thalassemia are amongst the most common monogenic diseases with tens of millions of patients globally. They arise from mutations in the beta-globin gene HBB and the only curative treatment is an allogenic hematopoietic stem cell transplant, which is complicated by limited matching donor availability, severe adverse reactions and excessive costs. Hemoglobinopathy symptoms can also be ameliorated with the increase of fetal hemoglobin (HbF), but pharmacological treatments are sub-optimal. However, naturally occurring beneficial alterations can lead to elevated levels of HbF in adulthood. When these benign Hereditary Persistence of Fetal Hemoglobin (HPFH) syndrome alterations are coinherited with a beta-hemoglobinopathy the disease can be much milder or even symptomless. Several HPFH alterations have been identified. Gene therapy and gene editing tools such as ZFNs, and CRISPR/Cas9 hold much promise for the treatment of genetic diseases and have been studied also for the treatment of beta-hemoglobinopathies. The first CRISPR/Cas9 clinical trials are also underway. Here, we have used the CRISPR/Cas9 - based genetic engineering as well as single base editing to produce beneficial HPFH mutations to hematopoietic cell lines, control and SCD patient hematopoietic CD34+ cells *in vitro*. After genetic editing and differentiation to hemoglobin- producing red blood cells, the cells show up to 35% production of fetal Hb. The differentiation capacity and the genetic profile of the cells show no significant changes. Funding: Academy of Finland, grants 308481, 286773

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P07.10B

Genome-wide association study identifies seven novel loci associating with circulating cytokines and cell adhesion molecules in Finns

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Introduction: Genetic factors modulate the inflammatory load, but the exact mechanisms are incompletely understood.

Methods: We performed a genome-wide association study (GWAS) on 16 circulating inflammatory phenotypes in Northern Finland Birth Cohort 1966 (NFBC1966, N=5,284). A subsequent meta-analysis was completed for 10 phenotypes available in a previous GWAS [1] adding up to 13,577 individuals in the study. Complementary association tests were performed to study the effect of the ABO blood types on soluble adhesion molecule levels.

Results: We identified seven novel and six previously reported genetic associations ($p<3.1x10^{-9}$). We observed three loci associating with soluble vascular cell adhesion molecule-1 (sVCAM-1) level, one of which is the *ABO* locus that has been previously associated with soluble E-selectin (sE-selectin) and intercellular adhesion molecule-1 (sICAM-1) levels [2-4]. Our findings further suggest that the blood type B associates primarily with the concentration of sVCAM-1 while the A1 subtype shows a robust effect on sE-selectin and sICAM-1 levels. The genotypes in the *ABO* locus associating with higher soluble adhesion molecule levels tend to associate with lower circulating cholesterol levels and lower cardiovascular disease risk.

Conclusions: The present results extend the knowledge about genetic factors contributing to the inflammatory load. Our findings suggest that two distinct mechanisms contribute to the soluble adhesion molecule levels at the *ABO* locus and that increased soluble adhesion molecule levels by itself may not increase risk for cardiovascular disease.

References: (1) Am J Hum Genet 2017;100:40-50. (2) PLoS Genet 2008;4:e1000118. (3) PLoS One 2012;7:

e51441. (4) Arterioscler Thromb Vasc Biol 2009;29:1958-67.

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P07.11C
DOCK8 deficiency; two patients with large deletions detected by Next-generation sequencing

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Dedicator of cytokinesis 8 (*DOCK8)* gene mutations lead a combined primary immunodeficiency which is first described as a new entity in the autosomal recessive Hyper IgE syndrome (HIES). Just like the HIES, these mutations cause elevated serum IgE, eosinophilia, frequent staphylococcal infections. Patients have increased risk for infections as well as autoimmunity and malignancy. DOCK8 gene spans 48 exons and deletions are the most common type of mutations. Here, we present 2 primary immune deficiency patients who are clinically diagnosed as DOCK8 deficiency. Both of the patients have consanguineous parents and laboratory results indicating DOCK8 deficiency. Ion AmpliSeq™ Primary Immune Deficiency Research Panel designed for sequencing 264 primary immune deficiency genes were used for mutation detection. After initial analysis of 264 genes filtering through minor allele frequency, variant location and variant effect no causative mutation was detected. Immune gene panel rechecked in both cases due to the strong clinical findings and large deletions were detected in both patients. First patient was found to have a homozygous deletion (c.(53+1_54-1)_(827+1_828-1)del) extending from 2^{nd} to 7^{th} exons and second patient have homozygous c.(53+1_54-1)_(3234+1_3235-1) deletion extending from 2^{nd} to 26^{th} exons.

We want to underline that large deletions may escape from routine filtering analysis and the suspected genes should be checked individually by an integrative genomic viewer. If deletions are the majority types of the mutation for some particular genes, this possibility should not be underestimated.

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P07.12D
Genetic variant rs16944 in IL1B gene is a risk factor for early onset sepsis susceptibility and outcome in preterm infants

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Introduction: Neonatal sepsis is progressive immunological process caused by invasion of microbial pathogens on the normally sterile tissue of infants. Despite the significant improvement of intensive neonatal care it is the most frequent cause of morbidity and mortality among preterm infants. Interleukin-1-beta (IL1B), is a proinflammatory mediator that appears very early in response to infection. The polymorphism *-511 G/A* in *IL1B* gene is located upstream of the transcriptional start site and influences its transcriptional activity. The aim of this study was to evaluate the relationships between rs16944 polymorphism in *IL1B* (-511 G/A) and susceptibility and outcome of early onset sepsis (EOS) in preterm infants.

Material and Methods: Our study included 471 preterm infants, 285 with EOS and 186 healthy premature infants. For all patients data about gender, gestational age, weight on birth (BMW), Apgar score determined in the 5th minute (AS5'), type of delivery and multiple pregnancies are collected. *IL1B -511G/A* genotypes were detected with custom real time genotyping assay.

Results: Frequency of AA *IL1B -511G/A* genotype is statistically significantly higher in EOS group (p=0.028). Genotype frequencies between EOS and control groups by recessive (GG+GA/AA) model also show significant differences (p=0.012). Logistic regression with weight on birth and gestational age as covariates confirmed significant association between *IL1B -511 G/A* genotypes and development of sepsis (B=-0.427, p=0.000). Also, in the EOS group AA genotype was significant predictor of lethal outcome (p=0.033).

Conclusion: *IL1B -511 G/A* polymorphism could be associated with early onset sepsis susceptibility and outcome in preterm infants.

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P07.13A
Genome-to-genome analysis of Epstein-Barr virus infection

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Epstein-Barr virus (EBV) is one of the most common viruses latently infecting humans. It is the cause of infectious mononucleosis and has oncogenic potential. Little is known about the potential impact of human genetic variation on inter-individual differences in response to EBV. The challenge of genetic determinant discovery explaining host susceptibility and response to EBV infection is compounded by pathogen genetic variation. We here use a genome-to-genome (G2G) strategy to search for genetic associations between paired human and EBV samples, which could highlight sites of host-pathogen genomic conflicts and shed light on pathogenesis.

We obtained and curated human genome-wide genotyping data and consensus EBV genome sequencing data from 266 HIV-infected individuals with elevated EBV plasma viral load (>2000 copies/ml). We performed a separate genome-wide association study (GWAS) for each viral variant using generalized linear mixed models, while controlling for human and EBV population structure.

We performed a total of 850 GWASs. No host-pathogen association survived the Bonferroni corrected G2G significance threshold of 10^{-11}. We observed four signals with $P<10^{-8}$, including an association between variants in the EBV gene BFRF1 and in the human gene *RSRC1*, which functions in spliceosome assembly and participates in multiple steps of mRNA splicing. Because EBV depends on host proteins for viral gene expression, changes in alternative splicing due to *RSRC1* variation could influence EBV pathogenesis by selecting specific BFRF1 escape mutations. The joint analysis of host and pathogen genetic variation can help uncover novel genetic influences on infectious diseases.

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P07.14B
Implementation of a new diagnostic strategy for familial erythrocytosis in Slovenia

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Introduction: Familial erythrocytosis is a rare congenital disorder defined by increased red blood cell number, haemoglobin and haematocrit. Variants in genes involved in oxygen sensing pathway (including *EGLN1*) or genes affecting haemoglobin oxygen affinity results in seven different types of familial erythrocytosis.

Materials and Methods: National diagnostic algorithm was developed to identify patients with familial erythrocytosis, among patients undergoing polycythemia vera testing over five-year period in Slovenia. Libraries were prepared according to the protocol for Illumina Nextera DNA Exome, enriched based on the protocol for Integrated DNA Technologies xGen Hybridization Capture of DNA Libraries and sequenced on MiniSeq sequencer. Identified variants were validated by Sanger sequencing.

Results: National diagnostic algorithm revealed that among 1054 polycythemia vera negative patients, only 81 had increased haemoglobin and haematocrit at least twice over a 2 month period. Further examinations pointed out seven families indicative for familial erythrocytosis. Targeted exome-sequencing identified heterozygous substitution rs61750991 in *EGLN1* gene in two affected but not in unaffected members of one family. The described diagnostic algorithm was also established within the in-house developed ViDis platform, enabling visualisation and sharing of medical algorithms (http://vidis.fri.uni-lj.si).

Conclusions: We successfully established diagnostic algorithm for familial erythrocytosis in Slovenia and facilitated its broad accessibility within medical society via ViDis platform. Implementation into clinical practice identified an *EGLN1* variant rs61750991 in one studied family, indicative for familial erythrocytosis type 3.

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P07.15C
Genotypic diversity observed within a large cohort of Armenian patients with late-onset familial Mediterranean fever

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Introduction: Familial Mediterranean fever (FMF) as an autoinflammatory disease, results from mutations in the *MEFV* gene mainly with an autosomal recessive mode of inheritance. The age of onset of FMF varies, with about 60% and 90% of patients experiencing their first attack before the age of 10 and 20 years, respectively. Hence, FMF with the first attack occurring at the age of ≥ 40 years (i.e. late-onset FMF) is rare and only a few small studies have addressed this disease subset.

Objectives: This work aimed at investigating the molecular genetic characteristics of Armenian patients diagnosed with late-onset FMF.

Methods: Genomic DNA isolated from 354 Armenian late-onset FMF patients were analysed for the 12 most common *MEFV* mutations plus SAA1 isoforms 1.1, 1.3 and 1.5 using multiplex PCR and reverse-hybridisation. Mutational spectra and resulting genotypes were then matched against the clinico-demographic profiles collected for these patients.

Results: Of all 354 patients, 194 (54.80%) were female and 160 (45.20%) were male. The following genotypes were significantly associated with the late-onset variant: M680I/E148Q (P=0.004), M694V/E148Q (P<0.001) and V726A/V726A (P=0.001). Of note, 12/354 (3.39%) patients were found to be homozygous for the M694V mutation.

Conclusions: Our data suggest that late-onset FMF is more prevalent in women and is of greater genetic diversity than previously reported. Further studies including late-onset FMF patients homozygous for *MEFV* mutation M694V are ongoing and may lead to the identification of novel disease-modifying mechanisms.

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P07.16D
Rare frameshift mutation in *SERPINA3* contributes to generalized pustular psoriasis

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Recent research in psoriasis has identified pustular manifestations as either Mendelian or oligogenic traits in contrast to the numerous associated SNPs in common plaque psoriasis. Autosomal-recessive mutations in *IL36RN* have been identified in 16-30% of patients with generalized pustular psoriasis (GPP), a rare, severe pustular psoriasis accompanied by multi-systemic inflammation. These mutations increase pro-inflammatory signaling in the IL-36 pathway. In exome sequences of 25 independent patients negative for *IL36RN*, we identified 2 patients with the

identical heterozygous frameshift variant in *SERPINA3*. We next sequenced the coding exons in further 54 GPP and 319 palmoplantar PP patients by Sanger without identifying further truncating variants. *SERPINA3* is an excellent functional candidate as it encodes serine protease inhibitor A3 which inhibits cathepsin G, a neutrophil-specific protease known to increase IL-36ß activity >500fold. *SERPINA3* expression in liver cell lines, fibroblasts and the epidermal cell line HaCaT could be stimulated by pro-inflammatory cytokines and dexamethasone. Protein amounts in sera of the two mutation carriers were in the lower normal range. By transfecting HaCaT cells with vectors containing the mutant or wildtype cDNA, we observed evidence for an unstable mRNA and lack of protein in the mutant. Immunohistochemical analyses revealed an increased staining of serpin A3 within upper epidermal layers in GPP, accentuated at the edge of psoriatic pustules. This close proximity to neutrophils suggests an interaction between serpine A3 and the neutrophilic enzyme cathepsin G. This interaction might be compromised by *SERPINA3* variants leading to more activation of pro-inflammatory IL-36ß.

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P07.17A

Differential expression of Type I IFN signaling pathway genes in Hepatitis C patients treated with Pegylated Interferon plus ribavirin

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Introduction: In Chronic Hepatitis C, 20% of patients will develop cirrhosis and may progress to hepatocellular carcinoma. Over the past years, many studies have tried to identify how IFN-λ and Type I Interferon pathway genes are expressed during treatment.

Materials and Methods: We evaluated the expression of genes of the type I IFN signaling pathway in 24 CHC patients treated with pegylated interferon plus ribavirin and further stratified according to rs12979860 polymorphism. Samples were evaluated at different time points (time 0, week 1 and week 12 during treatment and week 3 after treatment). Allelic discrimination was performed using Taqman Genotyping SNP assay (AHBKCW9, ThermoFisher). Expression levels for 30 Type I IFN genes was evaluated using Biomark HD System (Fluidigm). Kruskal-Wallis and Dunn´s post tests were used for comparisons.

Results: Most of the genes had elevated expression levels before treatment, except for *IFNA1, IFNAR* and *IFHI*. Overall, they had a decline in week 1 and a significant higher expression levels in later time points. In week 1 during treatment, CC homozygote patients for rs12979860 had lower gene expression levels, compared to patients that carried the risk allele T, this was observed for *IFI6, IFI16, IRF9* and *RIGI*. In contrast, relative mRNA expression for *IFH1* and *RNASEL* were subtle higher in patients with CC genotype.

Conclusions: Gene expression levels in Type I IFN signature genes was different according to time points evaluated and also when patients were stratified according to rs12989860 polymorphism.

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P07.18B

Analysis of the CD40 and CD40LG in Turkish Hyper IgM Syndrome: Mutation profile and description of six novel mutations

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Hyper IgM syndromes (HIGM) is a group of primary immune deficiency disorders characterized by defective CD40 signaling. Five types of Hyper IgM syndrome have been characterized: Hyper IgM syndrome type 3 (autosomal

recessive) (MIM: 606843) is characterized by mutations of the *CD40* gene and Hyper-IgM syndrome type 1 (X-linked) (MIM 308230), characterized by mutations of the *CD40LG* gene. Patients with HIGM syndrome are susceptible to recurrent and severe infections and in some types of HIGM syndrome opportunistic infections and an increased risk of cancer as well. The disease is characterized by decreased levels of immunoglobulin G (IgG) in the blood and normal or elevated levels of IgM. The aim of this study was to evaluate the spectrum of CD40 and CD40LG gene mutations in Turkish HIGM patients. We present a molecular analysis of 9 Turkish HIGM patients. All mutant alleles were identified, including 7 *CD40LG* mutations 4 of which were novel and 2 novel *CD40* mutations. *CD40LG* mutations were c.31C>T (p.Arg11Ter), c.755G>A (p.Gly252Asp), were previously reported whereas c.89 T>A (p.Val30Asp), c.446G>A (p.Ser149Asn), c.578T>G L193R (p.Leu193Arg) c.616_619delCTCA (L206EfsX35) mutations were novel. Novel *CD40* mutations were c.170_172delTAA and one stoploss mutation c.830_833delAGTG. Herein, we describe on 8 HIGM patients of Turkish origin and report five novel mutations in *CD40* and *CD40LG* genes.

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P07.19C
The role of rare genetic variation in the Interleukin-1 pathway

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The interleukin-1 (IL1)-pathway has a fundamental role in infection and inflammation. It comprises both IL1-agonists and -antagonists that together are responsible for maintaining a balance in innate immunological response. Common variants in underlying genes have previously been associated with infection, inflammation, cardiovascular disease and cancer, whereas the role of rare variants remains to be elucidated.

The highly-multiplexed targeted re-sequencing technology Molecular Inversion Probes (MIPs) enabled a genetic screen for the complete coding sequence of 48 genes of the IL1-pathway. Rare Variant Burden Analysis (RVBA) was performed on 520 healthy individuals for whom extensive immunophenotyping measurements are available.

We identified 221 variants, out of which 166 rare (gnomAD-Exome AF< 1%), in 41 genes. When considering individuals with 1% most extreme cytokine production, RVBA showed association with identical genes over different stimulations; e.g. in individuals with the highest IL6-producing macrophages, *IL1R2* is associated with LPS-, Candida- and MTB-stimulations. When using the continuous measurements, associated genes are unique per stimulation. More than 50% of the continuous associations become stronger in extreme phenotypes; e.g. IL6-production in whole blood stimulated with PHA is associated to *NCF4* continuously, in the corresponding 1% of individuals with lowest IL6-production this signal is magnified and even extended by associations with *NCF2* and *CYBA* (other ROS-production genes).

We show that distinctive rare genetic variation, identified by MIPs, influence cytokine responses to various stimuli in different cell-types in healthy individuals. We now plan to validate initial associations by functional follow-up and aim to expand this method to various IL1-mediated diseases.

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P07.20D
Identification of novel Non-MHC association loci/region in IgAD patients through MHC risk allele stratification and polygenic risk score analysis

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Introduction: Immunoglobulin A deficiency(IgAD) is the most common human primary immunodeficiency disease and has strong association with the major histocompatibility complex (MHC), however, its etiology remains unclear. We aimed to identify non-MHC region markers associated with

IgAD by defined genotypic subgroups of IgAD based on MHC susceptibility alleles.

Materials and Methods: Total of 10993 individuals were analyzed. Previously published MHC risk haplotypes were evaluated and the subjects were then stratified based on the MHC susceptibility alleles (*HLA*B0801-DRB1*0301-DQB1*0201/HLA-DRB1*0701-DQB1*0202/ HLA-DRB1*01-DQB1*0501*). Gene-based association analysis was performed to investigate the non-MHC association using approximately 540000 SNPs and 24000 genes. Additionally, polygenic risk score (PRS) pathway/gene set analysis was performed using 4760 pathway/genes set to infer the pathway involved in the respective subgroup.

Results: The presence of *HLA-DRB1*0301-DQB1*0201* (P=0.07) or *HLA-DRB1*0701*(P=0.72) alone was not sufficient to confer susceptibility to IgAD. In the cohort carrying at least one MHC risk allele, a protective allele, rs4097492, at *STXBP6*($P=7.63x10^{-9}$) was observed. CD40 ($P=6.89x10^{-5}$) was observed potentially associated with patients homozygous for the *HLA-B*0801-DRB1*0301-DQB1*0201* haplotype whereas *DHX38*($P=8.60x10^{-5}$), a novel inhibitor of protein phosphatase 4, shows a weak association with patients homozygous for *HLA-DRB1*01-DQB1*0501*. For patients lacking any risk alleles, seven gene regions were identified, including *TNFRSF13B*(TACI) ($P=1.12x10^{-4}$), with suggestive evidence of association with IgAD. Pathway PRS analysis shows that cohort carrying at least one *HLA-B*0801-DRB1*0301-DQB1*0201* haplotype have strong association with autoimmune and immune pathway while *HLA-DRB1*01-DQB1*0501* cohort having association with asthma pathway and IgA production.

Conclusions: Our findings suggest that the pathogenesis of IgAD may be different depending on the presence of selected MHC susceptibility haplotypes.

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P07.21A
Application of whole exome sequencing for patients with inherited platelet disorders

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Inherited platelet disorders (IPDs) are disorders consisting of thrombocytopenia (reduction in number of platelets), thromobocytopathy (defect in function of platelets) or a combination of these. Diagnosis is often hampered by the lack of specificity and correct validation of current hematological assays. In addition, these disorders exhibit a genetic heterogeneity with over 50 genes involved. This study reports the results of an upfront diagnostic strategy using whole-exome sequencing (WES) with a targeted analysis of a panel of 145 genes involved in thrombosis and hemostasis (besides platelet genes also other genes involved in coagulation and venous thrombosis embolism are analysed). Sixty-Six patients suspicious for an IPD were subjected to this WES approach. Fourteen patients (21%) harbored (likely) pathogenic variants that explained the clinical spectrum in these patients. Genes affected were *GP9*, *MYH9* (2 cases), *NBEAL2*, *P2RY12*, *RUNX1* (3 cases), *SLFN14* and *VWF* (2 cases); all involved in thrombocytopenia or thromobocytopathy. Additionally, 2 patients with a mutation in the *THPO* gene and 1 patient with a mutation in S*EPRINC1* were observed, both genes are known to be involved in venousvenous thromboembolic disease thromboembolic diseasethromboembolic diseasevenous thromboembolic disease. In 4 (6%) other patients only one heterozygous (likely) pathogenic variant of an autosomal recessive gene was observed. In 12 patients (18%) a variant of unknown significance (VUS/class 3) was observed. Further segregation studies within the family en functional studies are required to fully solve these cases. In conclusion, we found that WES is a powerful tool in genetically diagnosing patients with IPD.

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P07.22B
Uncovering the pathogen induced host-response on RNA level to aid genetic diagnosis of primary immunodeficiencies (PIDs)

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Primary immunodeficiencies (PIDs) constitute a group of diseases characterized by immune system dysfunction, that, due to its phenotypical and clinically heterogeneous nature, remains complex to diagnose. Consequently, leading to misdiagnosis and misclassification of patients in a clinical setting. Although whole exome sequencing (WES) has proven its significance as a diagnostic assay in PIDs, two-

thirds of all patients remains diagnosis-negative. As accurate identification of the underlying genetic defect is paramount to the clinical management of the disease, exploring alternative approaches to improve PID diagnosis is crucial. Given the relative ease of access to the affected tissue and *ex vivo* stimulations, we hypothesize RNA-sequencing holds the potential to reduce this diagnostic gap significantly.

To gain functional insight into the host-response, 3'mRNA-seq was performed on peripheral blood mononuclear cells (PBMCs) isolated from five healthy donors. Mimicking bacterial, viral, fungal and a more general immune response, PBMCs were exposed to specific immune stimulants, i.e. S.Auris, PolyI:C, C.Albicans and LPS, respectively, *in vitro* for 4 and 24 hours (n=50). Differentially expressed genes were identified by correcting for baseline expression in non-stimulated PBMCs. In turn, comparison of differential expression between conditions enabled us to unravel the core host-response, as well as pathogen specific responses in both the early and late context. Collectively, these insights not only aid our quest to identify novel PID candidate genes, i.e. genes of unknown function or undescribed immune function, it also assists WES-based variant filtering and prioritization. Ultimately, paving way to expand the current host-response series for unsolved PID patients.

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P07.23C
Clinical importance of rapid exome sequencing in patients with inborn errors of immunity

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In case of a severe inborn error of immunity a fast diagnosis is of vital importance and can change clinical decision making. In order to get a diagnosis in critically ill patients with a differential diagnosis for an inborn immunity disorder we make use of our in-house rapid whole-exome sequence workflow with a turnaround time of 1-3 weeks. Using this workflow we, so far, have analyzed the exomes of 14 patients using a primary immunodeficiency gene-panel (currently 386 genes). Depending on the clinical phenotype, another gene panel analysis and/or an analysis of the entire exome was additionally performed in 8 out of 14 cases. For 10 of the 14 cases trio-sequencing was performed to enable *de novo* analysis. The panel analyses resulted in a genetic diagnosis in 3 patients (21%). In these 3 patients we detected a homozygous (likely) pathogenic variant in the genes *MKL1*, *DHFR* and *PRF1*, respectively. The exome-wide analysis did not result in additional diagnoses. For the 3 solved cases the genetic diagnosis had direct impact on the clinical decision making. Medication was adjusted, specific dietary suggestions were provided for the *DHFR* case and the genetic diagnosis was instrumental in finding potential matching transplantation donors for the *PRF1* and *MKL1* cases. Also the diagnosis helped the parents in future family planning. We conclude that rapid exome sequencing can be of great added value for the diagnosis and treatment choice of patients with a severe inborn error of immunity.

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P07.24D
The impact of rare and low-frequency genetic variants in common variable immunodeficiency (CVID)

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Introduction: NGS (Next Generation Sequencing) have uncovered hundreds of common and rare genetic variants involved in complex and rare diseases such as immune deficiencies. However, these rare variants cannot be classified clinically vice versa those common variants only marginally contributes to disease susceptibility. In this study, we evaluated the multi-gene panel results of CVID patients and argued that if rare variants located in different genes could in fact play a more important role in disease susceptibility and/or etiology.

Material and Methods: We performed NGS from 103 patients' peripheral blood via a CVID-related 19 genes panel *(CARD11, CD19, CD81, ICOS, CTLA4, CXCR4, GATA2, ICOS, IRF2BP2, MOGS, MS4A1, NFKB1, NFKB2, PLCG2, TNFRSF13B, TNFRSF13C, TNFSF12, TRNT1* and *TTC37)*. Detected variants were evaluated and classified based on their impact, pathogenicity classification and population frequency as well as the frequency within our study group.

Results: Next generation sequencing revealed 112 different (total of 227) variants with under 10% population frequency in 103 patients which 22 (19.6%) were benign,

28 (25%) were likely benign, 5 (4.5%) were likely pathogenic and 2 (1,8%) were pathogenic. Moreover, 55 (49.1%) variants were classified as variant of uncertain significance. We also observed different variant frequencies when compared to population frequency databases.

Conclusion: Case-control data is not sufficient enough to unravel the genetic etiology of immune deficiencies. Thus, it is important to understand the incidence of two or more rare variants' coexistence for the possible key role in the pathogenesis of immune deficiencies.

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P07.25A
Unravelling the role of HSPE1 in regulatory T cell heterogeneity identified by a single-cell transcriptomic approach

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Introduction: Regulatory T cell (Treg) signature is composed of cell clusters of discrete states dispersed in a continuum gravitating around four different well-definable functional poles. The cell number and function of memory Treg cells is of key question, as these cells promote reproductive fitness during human pregnancy by reinforcing immune tolerance against fetal antigens. The aim of our study was to examine the role of HSPE1 in Treg differentiation and its influence on the functional states.

Methods: Single-cell Treg and naïve T cell sequencing data were obtained from 10x Genomics repository. Python based Scanpy toolkit was used for the analysis of single-cell data. Furthermore, qPCR-based gene expression of *HSPE1* in circulating PBMCs was evaluated.

Results: We identified 7 different cell clusters in the Treg cell population. We defined 4 in-house panels for the Treg cell subtype identification. Two clusters of memory Treg were identified. Among Treg cells, *HSPE1* shows a cluster-dependent expression pattern; the memory subtype expressing the highest levels. Furthermore, mass spectrometry showed the presence of HSPE1 in BeWo trophoblastic cell-derived extracellular vesicles (EVs). BeWo-derived EVs bound to CD4+ T cells and induced downregulation of IL6RA together with an increased IL-10 production.

Conclusion: By our in silico approach, we defined 4 gene panels which provide a good identification tool for the different Treg cells subtypes. Single-cell analysis shows a Treg cell subtype-specific signature of HSPE1 expression. Our results raise the possibility that BeWo-derived HSPE1 + EVs may be an inducing factor for Treg cell differentiation and memory Treg expansion.

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P07.26B
Reduced Severity of Collagen-induced Arthritis in Peptidylarginine deiminase knockout 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. To clarify the physiological function of PADI4 and PADI2 in RA, we used collagen-induced arthritis (CIA), known as a RA model mouse. We examined that localization of PAD4 and PAD2 protein was indicated by immunohistochemistry in CIA mice. We also measured expression of Padi genes and various inflammatory cytokines in immune cells by real-time TaqMan assay and ELISA, respectively. We generated PADI4−/− and 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. Furthermore, we examined that the clinical disease score and expression levels of Padi genes in PADI2−/− CIA mice. It appears that PADI4 and PADI2 enhance collagen-initiated inflammatory responses. This study was supported by Grants-in-Aid for Scientific Research (C).

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P07.27C
A systems genetics approach to study Candida susceptibility using single-cell RNA-seq

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Candida albicans infection of the blood stream, candidaemia, has mortality rates over 30% and is the most common invasive fungal infection in immunocompromised patients. Candidaemia is poorly understood and has poor treatment available. To better understand candidaemia response, we used single-cell RNA-seq (scRNA-seq) in 24 hour *Candida* stimulated and unstimulated peripheral blood mononuclear cells of six donors to identify cell-type-specific responses to Candida stimulation.

Differential expression (DE) analysis with MAST bulk-like scRNA-seq, shows high (97.3%) concordance with an independent bulk RNA-seq candida cohort. In addition, MAST was also run on 7 cell types to identify cell-type-specific DE effects. While the CD4+ T cells and natural killer (NK) cells have most DE genes (1459 and 1313, respectively), classical monocytes have the most distinct response. We are now able to show that the previously identified interferon pathway upregulation in candidaemia is consistently present throughout all immune cell types after stimulation with *Candida albicans*.

Subsequently, we did eQTL analysis on 72 stimulated and 75 unstimulated individuals with bulk RNA-seq data available, to identify response-specific eQTLs. However, we were unable to link specific cell types to these eQTLs when we overlapped the eQTL genes with the DE genes, likely due to small sample sizes in both datasets. In conclusion, we found no enrichment of stimulation-specific eQTLs in cell-type-specific DE genes, but were able identify cell-type-specific responses to *Candida albicans* exposure, revealing NK cells to have a stronger response than expected from previous studies.

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P07.28D

Targeted next-generation sequencing suggests novel risk loci in juvenile onset systemic lupus erythematosus

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Introduction: Childhood onset systemic lupus erythematosus (SLE) is associated with a more aggressive disease course and higher mortality risk than adult onset SLE. To identify genetic risk loci in juvenile onset SLE (jSLE) we performed DNA sequencing in a Swedish SLE cohort.

Materials and Methods: Coding and regulatory regions of 1853 genes were sequenced in 958 patients with SLE and in 1030 healthy individuals. 117 of the patients had jSLE (disease onset at age <18 years). Target capturing was performed with a Roche NimbleGen custom liquid capture library followed by Illumina HiSeq2500 sequencing.

Results: Single variant case-control association analysis revealed that 40 SNVs were associated with jSLE (FDR<5%). These 40 SNVs were enriched for missense variants (8% vs 1.8% for all SNVs). Two coding SNVs in the *NCF2* gene showed the strongest evidence of association to jSLE (rs17849502 and rs17849501, best P= 1E-10, OR=3.9, 95% CI[2.6-5.9]). This association exceeded the signal from the human leukocyte antigen (HLA) region in statistical significance (best HLA SNV was *TNXB* rs369580, P=6E-08, OR=2.5[1.8-3.5]). In order to further isolate the genetic contribution to jSLE, a case-case association analysis comparing juvenile and adult onset SLE cases was also performed. The two top loci from this analysis were *IL27* and *LIFR* (P<1E-05). Currently replication of these two loci is ongoing in an additional 100 jSLE cases.

Conclusion: Using targeted sequencing we have identified coding SNVs in novel candidate risk loci in jSLE,

highlighting differences in the genetic risk factors for childhood and adult onset SLE.

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P07.29A

Rapid identification of a bi-allelic *SPTB* mutation in a neonate with severe haemolytic anaemia and liver failure

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Introduction: Erythrocyte membrane defects, caused by deficiencies in membrane proteins ankyrin and spectrin, are an important cause of neonatal non-immune haemolytic anaemia. Heterozygous mutations in *SPTB*, encoding β-spectrin, cause autosomal dominant hereditary spherocytosis. Few cases of bi-allelic mutations have been reported, with severe consequences, including hydrops fetalis and fatal or near-fatal anaemia. Rapid genomic testing facilitates early diagnosis and informs management in critically ill patients. We describe rapid genomic diagnosis of a novel homozygous *SPTB* mutation in a case of severe prenatal-onset haemolytic anaemia with transfusion-dependence, and conjugated hyperbilirubinaemia with hepatosplenomegaly.

Methods: Clinical rapid trio exome testing was performed on DNA extracted from peripheral blood using Agilent Sureselect QXT CREv2 kit, followed by sequencing on Illumina NextSeq500.

Results: We identified a novel homozygous missense variant NM_001024858.3(*SPTB*):c.6119C>T (p.Thr2040Ile), located in the spectrin repeat region. Parents were both heterozygous for this variant. The time from sample receipt to result was 68hrs. Pretransfusion eosin-5-maleimide (E5M) staining in the proband was markedly reduced (ratio <0.6) and blood film showed marked spherocytosis including microspherocytes and nucleated erythrocytes. Both parents demonstrated mildly reduced E5M staining, with occasional spherocytes and elliptocytes seen in the maternal and paternal blood films respectively. The proband has life-threatening haemolytic anaemia with progressive liver failure, and early genetic diagnosis has facilitated hypertransfusion to suppress ineffective erythropoiesis and reverse hepatic dysfunction.

Conclusions: This case of severe prenatal haemolytic anaemia due to a homozygous *SPTB* mutation broadens the genotypic and phenotypic spectrum of spectrin deficiency and highlights the value of rapid early genomic diagnosis.

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P07.30B

A genome-wide association study of CM-SJS/TEN with SOC in Japanese population based on whole genome sequencing

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Introduction: Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) are immunologically mediated severe reactions of the skin and mucous membranes. Although previous genome-wide association studies identified the common SNPs as genetic risk factors of cold medicine related SJS/TEN with severe ocular complications (CM-SJS/TEN with SOC), little is known about the contribution of rare variants and structural variants (SVs) to development of CM-SJS/TEN with SOC.

Materials and Methods: In order to investigate impact of these variants as well as common variants, we conducted the whole genome sequencing (WGS) of 133 CM-SJS/TEN with SOC patients and 418 healthy control.

Results: WGS identified more than 21 million variants, of which 2.3 million were SVs. Genome-wide association test of these variants reproduced the associations of previously reported common variants on *HLA-A* and chromosome 16q12.1 loci. In addition, the novel associations of microsatellite polymorphism near *CDH12* gene and the aggregation of rare coding variants on *TRPM8* and *PARD3* genes were identified. *in silico* gene expression analysis revealed that the disease susceptibility alleles of *HLA-A* and *BRD7* affect gene expression levels at whole blood in GTEx database.

Conclusion: Majority of variants with significant association with CM-SJS/TEN with SOC were found among non-coding region illuminating the regulatory role of genetic variations on the development of CM-SJS/TEN with SOC.

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P07.31C
Genetic predictors of immune response to bacterial and viral stimuli in children

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Background: Little is known about variability of immune responses to microbial ligands between individuals, and over time. Within the setting of a population based birth cohort, we investigated genetic predictors of immune responses to bacterial and viral stimuli in children.

Materials and Methods: We measured cytokine responses (n=27) to stimuli including live viruses and bacteria (n=14) in peripheral blood mononuclear cell from children at ages 11 (n=306) and 16 (n=335) years. We sequenced exons, promoters and 3'- and 5'-UTRs of 364 immune response genes. After QC, 1705 SNPs (LD R^2 < 0.8 and MAF > 8%) and 328 differentially expressed cytokine-stimulus pairs at age 11, and 364 cytokine-stimulus pairs at age 16 were available for cytokine Quantitative Trait Loci (QTL) analysis using linear regression.

Results: A missense variant in Toll-Like receptor 1 (TLR1) rs5743618 was a cytokine QTL for IFN-gamma response to the TLR1/2 stimulus PAM3CSK4 at age 11 (p-value$_{FDRcorr}$=1x10^{-4}) and age 16 (p-value$_{FDRcorr}$=3.42x10^{-9}) years. The same variant was a cytokine QTL for IL-13 response to PAM3CSK4 at age 11 (p-value$_{FDRcorr}$=1x10^{-4}) and age 16 (p-value$_{FDRcorr}$=3.53x10^{-3}) and IL-2 response to LPS at age 11 (p-value$_{FDRcorr}$=0.031) and age 16 (p-value$_{FDRcorr}$=3.34x10^{-2}) years.

Conclusions: The missense variant rs5743618 in TLR1 was identified as the main cytokine QTL for IFN-gamma and IL-13 to TLR1/2 ligand PAM3CSK4 as well as IL-2 responses to LPS. This variant has recently been associated with asthma and allergic rhinitis in large-scale GWAS. The mechanisms linking immune responses to allergic disease needs to be established.

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P07.32D
Rare variants in antiviral response genes drive severe viral respiratory infections in children

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A robust and self-limiting immune response is required for clearance of viral respiratory infections. In rare cases, life-threatening infections may occur in previously healthy children. To uncover this susceptibility to severe disease we searched for rare genetic variants in 120 children requiring intensive care support upon infection by a respiratory virus. We used exome sequencing followed by protein network analysis to catalog rare coding variants and cluster them by known physical and functional associations. We identified potentially causal variants in 14 genes involved in proinflammatory response and viral nucleic acid detection, including *DDX58* and *IFIH1*, encoding RIG-I and MDA5, respectively. Both proteins share a common mechanism of RNA recognition and signal repression. In the absence of viral infection, each is maintained in an autoinhibited state, where the CARD effector domain and the ATP-binding helicase domain are masked by the C-terminal repressor domain (CTD). Binding of viral dsRNA at the CTD relieves repression and results in a proinflammatory cascade. Three loss-of-function variants in *IFIH1* were previously reported

for this cohort. A further four patients had helicase / ATP-binding domain variants in *IFIH1* and one patient with a rare CTD variant. Rare variants were found in *DDX58* affecting the RNA binding motif; one patient harboured a variant predicted to disrupt the ATP-binding helicase. In total, we identified 10 rare variants in 15 patients. We present a primary immunodeficiency resulting in extreme susceptibility to common respiratory RNA viruses, due to genetic variants in a common pathway that severely impairs viral recognition. SNSF Grant PP00P3157529

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P07.33A

Whole exome sequencing and functional studies for Fanconi anemia diagnostics

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Introduction: Fanconi anemia (FA) patients exhibit chromosome fragility, bone marrow failure, malformations and cancer susceptibility. FA is caused by point mutations and large deletions in 22 genes following 3 heritability patterns, making it diagnostics challenging.

Material and Methods: 68 FA patients with a positive chromosome fragility test were analyzed by WES. Copy number variations were evaluated by sequencing data analysis with Rstudio. To test *FANCA* missense variants, wtFANCA cDNA was cloned and variants were introduced by site directed mutagenesis, and tested for its ability to complement DNA repair defects of a FANCA-KO human cell line generated by TALEN technologies.

Results: using WES data, we identified 93.3% of mutated alleles including large deletions later confirmed by MLPA or SNPs arrays. We demonstrated pathogenicity of 3 FANCA missense variants and demonstrated that 2 *FANCA* variants reported in mutations databases as "affecting functions" are non pathogenic SNPs. Deep analysis of sequencing data revealed the actual mutations, highlighting the importance of functional analysis.

Conclusion: WES and proper bioinformatics analysis are sufficient to effectively characterize FA patients regardless complementation group, type of mutations, mosaic condition, and DNA source.

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P08 Intellectual disability

P08.01B

***CNOT2* as the critical gene for phenotypes of 12q15 microdeletion syndrome**

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[Background]Chromosome 12q15 microdeletion is characterized by intellectual disability and dysmorphic facial features. Recently, the smallest region of overlap (SRO) in 16 previously reported patients was used to define three candidate genes for the 12q15 microdeletion syndrome: *CNOT2*, *KCNMB4*, and *PTPRB*. The relative contributions of these three genes have not yet been delineated. Herein, we document a patient with a microdeletion of the chromosomal 12q15 region and re-define the SRO. [Clinical Report]The patient was a 12-year-old female with intellectual disability and multiple structural abnormalities including cleft lip and palate and 2-3 toe syndactyly. She exhibited dysmorphic facial features such as upslanting and short palpebral fissures, micrognathia, low-set ears, and hypoplastic antihelix. [Molecular Analysis] A microarray analysis showed a *de novo* 1.32-Mb deletion within 12q15 that included *CNOT2*, a member of the CCR4-NOT complex that regulates gene expression by regulating transcription and mRNA degradation, and 14 other genes.

Results and Discussion: Remapping of the 12q15 deletion region in the 16 previously reported patients together with that in the newly identified patient indicated that *CNOT2* is the only gene that is commonly deleted. In conclusion, these findings suggest that *CNOT2* is the prime candidate for the neurological phenotypes of the 12q15 microdeletion syndrome.[Funding] This study was supported by Ministry of Health, Labour and Welfare (Grant Number: Research on Rare and Intractable Diseases) and Japan Agency for Medical Research and Development (Grant Number: JP18ek0109301).

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P08.02C

Bi-allelic pathogenic variants in the lanosterol synthase gene *LSS* involved in the cholesterol biosynthesis cause alopecia with intellectual disability, a rare recessive neuroectodermal syndrome

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Purpose: Lanosterol synthase (*LSS*) gene was initially described in families with extensive congenital cataracts. Recently, a study has highlighted *LSS* associated to hypotrichosis simplex. We expanded the phenotypic spectrum of *LSS* to a recessive neuroectodermal syndrome formerly named Alopecia with mental retardation (APMR) syndrome. It is a rare autosomal recessive condition characterized by hypotrichosis and intellectual disability (ID) or developmental delay (DD), frequently associated with early onset epilepsy and other dermatological features.

Methods: Through a multi-center international collaborative study, we identified *LSS* pathogenic variants in APMR individuals either by exome sequencing or *LSS* Sanger sequencing. Splicing defects were assessed by transcripts analysis and minigenes assays.

Results: We reported 10 APMR individuals from 6 unrelated families with bi-allelic variants in *LSS*. We additionally identified one affected individual with a single rare variant in *LSS* and an allelic imbalance suggesting a second event. Among the identified variants, two were truncating, seven were missense and two were splicing variants. Quantification of cholesterol and its precursors did not reveal noticeable imbalance.

Conclusion: In the cholesterol biosynthesis pathway, the lanosterol synthase leads to the cyclization of (S)-2,3-oxidosqualene into lanosterol. Our data suggest *LSS* as a major gene causing a rare recessive neuroectodermal syndrome.

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P08.03D

Genotype-phenotype correlation in 11 Angelman-like syndrome patients with new molecular diagnosis

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Introduction: Approximately 10% of patients with a clinical diagnosis of Angelman syndrome (AS) remain without a molecular diagnosis (AS-like). Whole-exome sequencing in a cohort of 17 AS-like patients led to the identification of 11 pathogenic/likely pathogenic *de novo* variants in 10 genes involved in neurodevelopment disorders, not previously associated with AS. Clinical re-evaluation was performed in order to establish the genotype-phenotype correlation in the molecularly diagnosed patients.

Materials and Methods: The clinical characteristics of the 11 patients were reviewed for the presence of the consistent and frequent AS clinical features. Additional distinctive clinical features were collected and compared with the clinical phenotypes reported in the literature in patients carrying pathogenic variants in the identified genes.

Results: Clinical re-evaluation showed that the 11 patients meet the consistent and almost all of the frequent features of AS. The additional clinical findings in patients carrying variants in *SMARCE1, KIF1A, SYNGAP1* and *SLC6A1* genes fit with the phenotypes reported in patients with pathogenic variants in the same genes. On the other hand, patients carrying variants in *SATB2, ASXL3, SPTAN1* and *LAS1L* genes present some but not all the consistent features attributed to these genes.

Conclusion: Molecular heterogeneity in AS-like patients, together with the presence of distinctive clinical features in some of them, indicates that AS-like is made up by different clinical entities overlapping phenotypically with AS, making them difficult to distinguish.

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P08.04A

When high incidence and high genetic heterogeneity lead to different genetic aetiologies within one family: a case of two sibs with neurodevelopmental disorders and *de novo* variants in one known and one candidate gene

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We report on a family with two affected patients, a 13-year-old girl (P1) with severe intellectual disability (ID), autism, agenesis of corpus callosum, abnormal EEG findings, hearing loss, eye defect and short stature, and her brother (P2) with congenital chylothorax and hydrops who died 36 hours after birth.

Exome sequencing identified in P2 a *de novo PTPN11* variant NM_002834.4:c.417G>C p.(E139D) listed as pathogenic in ClinVar and causing Noonan syndrome, which was consistent with the clinical findings. There were no clear-cut findings in P1, with a possible exception of a *de novo* frameshift *ARGLU1* variant NM_018011.4: c.695delAAGA p.(K232Ffs*6). *ARGLU1* encodes arginine- and glutamate-rich protein 1 which may have a transcriptional regulatory role. *ARGLU1* has not been associated with ID yet but is among possible candidate genes. The gene is intolerant to loss-of-function variation (3/20 observed/expected variants in gnomAD). Several *de novo ARGLU1* variants (gene deletions and a frameshift) have

been identified in a large candidate gene screen, and the same variant as that of P1 was *de novo* in one patient of the DDD cohort. The variant is located in the last exon but truncates the glutamate-rich domain, which may alter protein function. Phenotype information on previous patients is limited but suggests overlap with P1.

Our study supports the notion that due to the high incidence of neurodevelopmental disorders and their huge genetic heterogeneity affected siblings often have different genetic aetiologies. Additional patients and analyses are needed to confirm or exclude *ARGLU1* as a new ID gene.

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P08.05B
Lethal neonatal stiffness and immobility caused by biallelic variations in *ATAD1*

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ATAD1 encodes for thorase, a protein which regulates the surface-expression of AMPA receptors. Thus, thorase plays an essential role in neurotransmission. Biallelic loss-of-function or activating *ATAD1* mutations have been recently described in six patients from three different families. They presented with severe encephalopathy characterized by neonatal stiffness, limited or absent mobility, poor eye contact, feeding difficulties and respiratory distress.

Here, we report on two novel infants with this congenital immobility and hypertonia syndrome. Patient #1 needed intensive cares from birth because of secondary neonatal respiratory distress. He had extreme stiffness of limbs and axial hypotonia, immobility, absence of eye contact, limited responsiveness to stimuli and weak sucking. He had epileptic seizures at 5 months and died at 6 months. Patient #2 had a similar disease course, except that he had no epilepsy. He died at 4 months of cardio-respiratory failure. Both patients had null auditory evoked potentials.

Whole-exome sequencing (WES) in patient #2 revealed the previously reported c.1070_1071del p.(His357Argfs*15) *ATAD1* homozygous variant. We reinterpreted WES data obtained five years ago for patient #1, previously interpreted as normal, which highlighted the novel c.383G>T p.(Gly128Val) homozygous variant in *ATAD1*.

The phenotype of these two patients was close to that of published patients, associating neonatal stiffness with immobility. We confirm the auditory involvement reported once. The retrospective diagnosis of our patient #1 illustrates that *ATAD1* mutation is associated with a recognizable clinical presentation, which is critical for genetic counseling and potential targeted therapy.

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P08.06C
New candidate genes in autism spectrum disorder

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Introduction: Autism spectrum disorder (ASD) is a well-known highly genetic heterogeneous entity.

Materials and Methods: In the context of an International project aiming at identifying the genetic causes of ASD (Autism Sequencing Consortium, Mount Sinai), we performed a whole exome sequencing (WES) analysis in a cohort of 100 ASD patients, not harboring CNVs, attending the Medical Genetics Unit of Siena.

Results: Mutations in already known ASD-related genes were detected in 11% of cases, while either de novo or inherited mutations in novel genes were identified in 21% of cases. The novel identified genes can be pooled in three categories: i-genes for which causative mutations have not

been previously reported (eight genes), ii-genes for which a likely association with ASD has been previously reported in a single case (eg DRP2 etc) and iii-genes whose mutations have been previously associated with intellectual disability (ID) in only one (eg BCORL1 etc) or a few families (eg BRD4 recently associated with a Cornelia De Lange-like phenotype etc).

Conclusions: Overall, our extensive approach goes beyond safely confirming the pathogenic role of ASD/ID candidate-genes and allows establishing the novel role of ID-related genes in ASD. It delineates the impact of new genes across neurodevelopmental dimensions, providing important new insights into functional pathways involved in heterogeneous ASD phenotypes.

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P08.07D

Mutations in *DLL1* cause variable neurodevelopmental disorders

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The evolutionarily conserved Notch signaling pathway operates in many different developmental, homeostatic and disease processes. *In vivo* and *in vitro* studies have shown an important role of the Notch ligand DLL1 in the development of the nervous system and somites, among others.

We identified 14 individuals from ten unrelated families with heterozygous *DLL1* variants by exome sequencing. The most common features in our cohort were intellectual disability, autism spectrum disorder, seizures and variable brain malformations. Four patients had scoliosis including one with a vertebral segmentation defect. We identified six nonsense variants, two splice site variants, one missense variant affecting a highly conserved cysteine in the DSL domain, and one 122 kb deletion containing *DLL1*. Analysis of the splice site variant NM_005618.4:c.54+1G>A showed an in-frame insertion of 12 bp near the sequence coding for the signal peptide, possibly affecting proper localization of the mutant DLL1 protein. The features in our cohort resemble those of patients with overlapping terminal deletions of 6q27 encompassing *DLL1*, supporting the previous hypothesis that haploinsufficiency of *DLL1* is causative of the phenotype in this deletion.

In conclusion, we identified heterozygous mutations in *DLL1* in a cohort with a variable neurodevelopmental phenotype and other multi-system features. Our clinical and molecular data support haploinsufficiency as a mechanism for the pathogenesis of *DLL1*-related disorders and illustrate the importance of DLL1 in human brain development.

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P08.08A
Exploring the phenotypical spectrum of BRD4 defects

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Bromodomain Protein 4 (BRD4) is a member of the bromodomain protein family involved in binding to hyperacetylated genomic regions of promotors and enhancers. BRD4 mediates CDK9 activity to influence transcription elongation by RNA polymerase II and thus regulates gene expression, cell differentiation and cell cycle. Recently, frameshift and missense variants were reported with a Cornelia de Lange-like phenotype. In three independent families we identified 2 missense and 1 frameshift heterozygous *de novo* variants in *BRD4* (c.1856G>T; c.2513A>T; c.2728delC). The individuals with the c.1856G>T and the c.2728delC variants presented with intellectual disability and Cornelia de Lange-like facial gestalt. Short stature was present in all but the individual with the frameshift variant. Protein structure modelling indicates that variant c.1856G>T (p.Ser619Iso) likely affects binding of BRD4 to its ligands. Expression analysis showed that both missense variants, but not the frameshift variant, lead to a reduced expression indicating a loss-of-function effect of all three identified variants. Immunofluorescence analysis from cells with the missense variants confirmed a reduced cellular growth and smaller cell size compared to controls. Transcriptional profiling to assess the effect of BRD4 on known effector proteins highlighted a complex dysregulation in patient and CRISPR/Cas9 meditated BRD4 defect cell lines. We expand the clinical spectrum of mutations in BRD4 from idiopathic short stature without distinct facial gestalt to a Cornelia de Lange-like phenotype and propose a loss-of-function effect of *BRD4* mutations. The variability of the phenotype might in part be explained be different effects on target effector genes.

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P08.09B
Truncating *CHD8* variants cause a Sotos-like syndrome with autism

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Variants in the chromodomain helicase DNA-binding protein 8 (CHD8) have been associated with intellectual disability (ID), autism spectrum disorders (ASD) and overgrowth and *CHD8* is one of the causative genes for OGID (overgrowth and ID). However, the phenotypic spectrum of individuals heterozygous for *CHD8* truncating variants has not been clearly defined. We investigated 26 individuals with *CHD8* protein truncating variants (PTVs), including 10 previously unreported patients, and found a

pattern of common features: macrocephaly (76%), tall stature (65%), developmental delay and/or intellectual disability (DD/ID, 81%), autism spectrum disorders (ASD, 81%), sleep difficulties (48%), gastrointestinal problems (42%), and distinct facial features. The gestalt is reminiscent of Sotos and Weaver syndromes. Most of the individuals in our cohort had moderate-to-severe intellectual disability, and some had regression of speech (37%), seizures (27%) and hypotonia (27%), and two individuals were also non-ambulant. Most individuals had ASD (81%), similarly to previous studies, but we found a higher incidence of comorbid neurodevelopmental delay/impairment. Our study shows that haploinsufficiency of *CHD8* is associated with a Sotos-like syndrome with pronounced autistic traits.

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P08.10C

Delineation of the clinical phenotype caused by *de novo CLTC* variants

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Introduction: Pathogenic variants in the *CLTC* gene have been reported in 14 patients with intellectual disability (ID) with or without epilepsy.

Methods: We describe 13 novel patients with a likely pathogenic *de novo CLTC* variant, identified by using diagnostic exome sequencing or an ID gene panel.

Results: All individuals presented with intellectual disability (ID), ranging from mild to moderate/severe, with or without additional neurologic, behavioral, craniofacial, ophthalmologic and gastrointestinal features. The severe end of the clinical spectrum, including severe ID, epilepsy, microcephaly and hypoplasia of corpus callosum was more frequently observed in the group of individuals with missense and in-frame variants than in those with nonsense and frameshift variants, although this difference was not significant (Fisher's Exact Test; p-value>0.0125). A 3D model of the CHC1 protein showed that the *de novo* missense *CLTC* variants do not cluster in 3D dimensional proximity. However, these variants may affect the interaction with other clathrin heavy and light chains as CHC1 interacts with three light chains to form clathrin. The nonsense and frameshift variants are all predicted to result in nonsense-mediated mRNA decay.

Conclusion: Taken together, these results suggest that missense and in frame variants exert a dominant negative effect (antimorph), whereas the nonsense and frameshift variants would result in haploinsufficiency (hypomorph). Consequently, the wide phenotypic variability observed in *CLTC*-related ID seems to be associated with allelic heterogeneity.

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P08.11D

Four further patients with bi-allelic CNTNAP2 aberrations

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Biallelic defects in CNTNAP2 gene were previously described in an autosomal recessive disorder with severe intellectual disability (ID), epilepsy and cortical dysplasia. To date, 25 affected individuals have been reported. Most of patients have severe ID with absent or limited expressive speech and limited verbal comprehension. Only two patients with moderate ID and able to use sentences were reported. We described four children from three families with novel homozygous or compound heterozygous deleterious CNVs and mutations in CNTNAP2 gene. Motor milestones were mildly delayed in all patients, with a walking age between 22 and 30 months. All patients had ID. Two patients had severe ID with complete lack of speech. Two unrelated patients had mild ID with preserved speech and good verbal comprehension. One of them was able to write and read, while his brother had severe intellectual disability with no language. Epilepsy occurred in all patients, with an age at onset between 3 and 24 months. One patient was seizure free for 4 years. All patients, except one, had cortical dysplasia. No specific facial dysmorphism was noted. Birth parameters and postnatal growth were in the normal range. In conclusion, we reported four individuals from three families with biallelic aberrations in CNTNAP2 causing ID and additional features such as epilepsy and cortical dysplasia. In contrast to previous reports, we observed moderate ID with preserved speech and verbal comprehension in two children suggesting clinical variability even in a same family.

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P08.12A

Novel *de novo* 2q14.3 deletion disrupting *CNTNAP5* in a girl with intellectual impairment and microcephaly

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Introduction: Reports of small 2q14.3 deletions including *CNTNAP5* are rare and the clinical effect is currently unknown. Here we present a case of an 838kb *de novo* 2q14.3 deletion involving multiple exons of *CNTNAP5* in a child with developmental delay and microcephaly.

Materials and Methods: A 9-year-old girl with an intellectual disability and microcephaly (OFC < 0.4th percentile) was seen in the genetics clinic. The girl had particular difficulties with visual-spatial tasks and she had very low working memory and processing speed. An 850 kb SNP array (Illumina Human CytoSNP-12 Beadchip) was done which identified the deletion. No other copy number variations were identified and detailed investigations for an alternative cause were all normal. Parents were of normal intelligence.

Results: An 838kb *de novo* 2q14.3 deletion was identified (chr2: 125,204,264-126,042,867; hg19). There are few reports of deletions involving *CNTNAP5* but all have involved multiple other genes or copy number variants. This is the first reported case of a *de novo* deletion disrupting *CNTNAP5* in an individual with intellectual disability and microcephaly and no other CNVs identified. CNTNAP5 is a member of the neurexin family of multidomain transmembrane proteins involved in cell adhesion and intercellular communication, leading to our hypothesis that mutations in *CNTNAP5* result in abnormalities of neurodevelopment.

Conclusion: We hypothesise that *CNTNAP5* is an important gene in neurodevelopment and disruption of *CNTNAP5* causes neurodevelopmental disability. 2q14.3 deletions involving *CNTNAP5* may constitute a new deletion syndrome.

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P08.13B

Linked homozygous CRADD and USP44 variants in intellectual disability

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Background: The global prevalence of intellectual disability is estimated to be around 1%; however, the molecular basis remains unknown in most cases. We studied a consanguineous Pakistani kinship with severe intellectual disability, mild lissencephaly, brain atrophy and progressive decline in cognitive skills to identify the genetic basis of the disease.

Materials and Methods: Homozygosity mapping was performed and exome sequence file of one affected individual was investigated for candidate variants.

Results: In a 3.9-Mb region of shared homozygosity, we detected *CRADD* c.2T>G variant and novel *USP44*

c.873_884del and c.886delA variants. *CRADD* variant alters the initiation codon (AUG◊AGG; p.(M1?) or p.(M1A)), and it is extremely rare (highest MAF=3.28E-05). *CRADD* variant leads to premature translational termination.

Discussion: Biallelic damaging variants in *CRADD* cause recessive mental retardation 34 (MRT34) with mild to moderate intellectual disability (ID) and in some cases additional lissencephaly and megalencephaly. Only one ID family has been reported with an *USP44* variant, which is a deletion similar to ours. Phenotype of our patients, severe intellectual disability and mild lissencephaly but no seizures, is different from phenotypes of patients with *CRADD* or *USP44* mutations alone and is not as severe as would be expected of an additive effect.

Conclusion: Familial linked homozygous variants are very rare, and we found such variants in *CRADD* and *USP44* in two cousins with severe intellectual disability and mild lissencephaly. This study also highlights that whole exome sequencing rather than candidate gene approach can uncover a novel molecular basis for a disorder.

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P08.14C

Spatially clustering de novo variants in *CYFIP2*, encoding the cytoplasmic FMRP interacting protein 2, cause intellectual disability and seizures

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CYFIP2, encoding the evolutionary highly conserved cytoplasmic FMRP interacting protein 2, has previously been proposed as a candidate gene for intellectual disability and autism because of its important role linking FMRP-dependent transcription regulation and actin polymerization via the WAVE regulatory complex (WRC). Recently, de novo variants affecting the amino acid p.Arg87 of *CYFIP2*

were reported in four individuals with epileptic encephalopathy. We here report 12 independent patients harboring a variety of de novo variants in *CYFIP2* broadening the molecular and clinical spectrum of a novel *CYFIP2*-related neurodevelopmental disorder. Using trio whole-exome or -genome sequencing, we identified 12 independent patients carrying a total of eight distinct de novo variants in *CYFIP2* with a shared phenotype of intellectual disability, seizures, and muscular hypotonia. We detected seven different missense variants, of which two occurred recurrently (p.(Arg87Cys) and p.(Ile664Met)), and a splice donor variant in the last intron for which we showed exon skipping in the transcript. The latter is expected to escape nonsense-mediated mRNA decay resulting in a truncated protein. Despite the large spacing in the primary structure, the variants spatially cluster in the tertiary structure and are all predicted to weaken the interaction with WAVE1 or NCKAP1 of the actin polymerization regulating WRC-complex. Preliminary genotype–phenotype correlation indicates a profound phenotype in p.Arg87 substitutions and a more variable phenotype in other alterations. This study evidenced a variety of de novo variants in *CYFIP2* as a novel cause of mostly severe intellectual disability with seizures and muscular hypotonia.

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P08.15D

Diagnostic whole exome sequencing identifies new causative variants in 1000 cases with intellectual disability

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We performed diagnostic Whole Exome Sequencing (WES) in 1037 cases with intellectual disability (ID) and filtered for pathogenic variants as well as pathogenic CNVs in genes previously reported in ID. In 234 cases we ran Trio analysis; in another 79 cases we sequenced more than one affected individual per family. With this approach we could identify pathogenic variants or CNVs in 31% of the cases and prioritize candidate variants of unclear significance in another 17.6% of the cases.

About 66% of the pathogenic variants occurred *de novo* or were transmitted in an autosomal dominant manner, while 21% of the variants were autosomal recessive. Only a minority of 11% was X-chromosomal and less than 1% occurred in mitochondrial DNA.

Among these we identified several cases with pathogenic variants in *HNRNPH2, NBEA*, *PUS3*, *RHOBTB2*, *TBCK* and *TBC1D23*, genes that have been associated with ID very recently. Besides identifying pathogenic variants in genes previously associated with ID, we were also able to identify 22 new candidate genes such as *BCL11B*, *FBXO11* and *KMT2E*.

Taken these results together we could reveal causative variants in about 31% of cases and identify potentially causative variants in another 17% of cases in a study comprising more than 1000 individuals with intellectual disability.

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P08.16A
Language impairment with a partial duplication of *DOCK8*

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In this talk we will report on a boy with a microdeletion in 8p23.1 (arr[hg19] 8p23.1 (7169490-7752586) and a microduplication in 9p24.3 (arr[hg19] 9p24.3 (266045-459076). Our proband exhibits a moderate language delay, mostly affecting the expressive domain, as well as cognitive delay and behavioural deficits, including attentional problems and aggressiveness. Whereas the microdeletion in chromosome 8 is reported as benign, the microduplication in chromosome 9 is hypothesized as pathogenic, affecting the body of the gene *DOCK8*. We will discuss the role of this gene in brain development and function, focusing on the functional links of the DOCK8 protein with CDC42, in turn associated to nearly 30 candidates for language disorders and/or language evolution, including SLIT1, SLIT2, and ROBO1, which play a key role in the externalization of language (speech). We will also present results of a targeted whole transcriptome sequencing analysis (RNA-seq) aimed to determine changes in the expression levels in the blood of *DOCK8*, its functional partners with a known role in language development, impairment, and/or evolution (*ABL1, DCDC2, DOCK4, MAPK1, MET, ROBO1*, and *SLIT2*), and several other robust candidate genes for language disorders and/or language evolution (*AUTS2, BAZ1B, BMP2, CMIP, CNTNAP2, DLX1, ELP4, FLNA, FOXP1, FOXP2, GRIN2A, POU3F2, RUNX2*, and *SOX9*). We will conclude that the phenotype exhibited by our proband might result from a severe deficit in working memory (mostly impacting on language structure and use), seemingly resulting from a reduced dosage of DOCK8 and the subsequent alteration of a CDC42-regulated network important for language development.

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P08.17B
Biallelic variants in *DYNC1I2* cause syndromic microcephaly with intellectual disability, global developmental delay and dysmorphic facial features

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Cargo transport along the cytoplasmic microtubular network is essential for neuronal function, and cytoplasmic dynein-1 is an established molecular motor critical for neurogenesis and homeostasis. We performed whole exome sequencing, homozygosity mapping, and chromosomal microarray studies in five individuals from three independent pedigrees and identified likely pathogenic variants in *DYNC1I2* (Dynein Cytoplasmic 1 Intermediate Chain 2), encoding a component of the cytoplasmic dynein 1 complex. In a consanguineous Pakistani family with three affected individuals presenting with microcephaly, severe intellectual disability, cerebral malformations and dysmorphic facial features, we identified a homozygous splice donor site variant (NM_001378.2:c.607+1G>A). We report two

additional cases with similar neurodevelopmental deficits and craniofacial features who harbor deleterious variants: an individual bearing a p.(Tyr247Cys) change in trans with a 374 kb deletion encompassing *DYNC1I2*; and an unrelated case harboring compound heterozygous variants p.(Gln290*) and p.(Tyr247Cys). F0 zebrafish larvae with CRISPR/Cas9 gene disruption or transient suppression of *dync1i2a* displayed significantly altered craniofacial patterning with concomitant reduction in head size. We monitored cell death and cell cycle progression in *dync1i2a* zebrafish models and observed significantly increased apoptosis, likely due to prolonged mitosis caused by abnormal spindle morphology, offering initial insights into the cellular basis of microcephaly. Additionally, complementation studies in zebrafish demonstrate that the p.(Tyr247Cys) attenuates gene function, consistent with protein structural analysis. Our genetic and functional data indicate that the *DYNC1I2* dysfunction likely causes an autosomal recessive microcephaly syndrome, and highlight further the critical roles of the dynein-1 complex in neurodevelopment.

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P08.18C
9q34.3 microduplications lead to Neurodevelopmental Disorders through *EHMT1* overexpression

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Both copy number losses and gains occur within subtelomeric 9q34 region without common breakpoints. The microdeletions cause Kleefstra syndrome (KS), whose responsible gene is *EHMT1* (euchromatin histone methyltransferase 1). A 9q34 duplication syndrome (9q34DS) had been reported in literature, but it has never been characterized by a molecular point of view. At the best of our knowledge, we report on the two patients carrying the smallest 9q34.3 duplications containing *EHMT1* as the only relevant gene. We compared them to 21 described patients carrying 9q34.3 duplications encompassing the entire gene and extending within 3 Mb~. By surveying the available clinical and molecular cytogenetic data, we could find out that similar neurodevelopmental disorders (NDDs) were shared by patients carriers of even very different sized duplications. Moreover, some facial features of the 9q34DS were more represented than those of KS. However, an accurate *in silico* analysis of the genes mapped in all the duplications allowed us to support *EHMT1* as being sufficient to cause a NDD phenotype. Wider patient cohorts are needed to disentangle whether the rearrangements have full causative role or simply confer the susceptibility to NDDs and possibly to identify the cognitive and behavioral profile associated to the EHMT1 increased dosage.

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P08.19D
Epha7 haploinsufficiency is associated with neurodevelopmental delay

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Interstitial 6q15-q16.1 deletions are rare and few cases have been reported in the literature. Genotype-phenotype correlation is complex because of incomplete penetrance and variable expressivity, various size and breakpoints of the 6q deletions. We report 6 additional patients from two families with 6q16.1 deletion encompassing or disrupting *EPHA7* gene. All patients presented a neurodeveloppemental disorder. SNP array analysis of family 1 (patient 1 to 5) showed a 4,5 Mb deletion at 6q16.1 disrupting exons 10-17 of *EPHA7* (OMIM *602190) in all affected children and also inherited from the affected father. For family 2 (patient

6), SNP array analysis revealed a 3,1 Mb deletion at 6q15q16.1 encompassing only one OMIM gene: *EPHA7*. The ephrin receptor A7 (*EPHA7*, mapped in 6q16.1) encode a member of ephrin receptor subfamily of the protein-tyrosine kinase family. EPH and EPH-related receptors have been implicated in mediating developmental events, particularly in the nervous system. EphA7 play a role in cortical domains formation, determine brain size and shape, and is involved in development of the central nervous system. We provide further evidence for *EPHA7* haploinsufficiency role in neurodevelopmental disorder and delineate clinical phenotype. Interestingly, one patient (patient 3) had a 6q16.1 deletion encompassing *EPHA7* and a 1,4 Mb 7q11.23 microdeletion associated with more severe symptoms than those observed in Williams-Beuren syndrome. *EPHA7* deletion could also contribute to the most severe neurodevelopmental phenotype as a second hit.

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P08.20A

A deleterious synonymous *SLC25A12* variant in a boy with severe epileptic encephalopathy

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Background: *SLC25A12* encodes a mitochondrial membrane protein ARALAR/AGC1 that contributes to the exchange of aspartate/glutamate. Biallelic pathogenic variants lead to decreased availability of N-acetyl aspartate in the brain, leading to early infantile epileptic encephalopathy (OMIM #612949) with global cerebral hypomyelination, developmental regression, apnea, seizures, and muscular hypotonia.

Case presentation: Here we report on a boy with profound developmental and motor delay, drug-resistant epilepsy starting at 7 months and recurrent episodes of status epilepticus. Trio-exome sequencing revealed the compound heterozygous variants in *SCL25A12;* c.225del; p.(Glu76-Ser*fs**17) and c.1747C>A; p.(=), which are absent in public databases. As the phenotype was overlapping and the synonymous variant was predicted to alter the splicing, we performed mRNA analysis on blood of the patient that revealed a loss of full-length cDNA. Western blot on muscle biopsy of the patient confirmed these results. Brain MRI including MR-spectroscopy further supported the genetic findings since choline/myoinositol were found to be elevated in the medullary layer, whereas NAA was reduced.

Conclusion: The clinical presentation as well as characteristic MR-spectroscopy pattern combined with the loss of mRNA/protein expression in patient´s tissues confirmed the pathogenicity of both variants. Thus, we could set the diagnosis of EIEE39. However, at our lab and as far as we know at other laboratories, synonymous (and many splicing) variants are not well characterized. Bioinformatics tools for splicing are often not well integrated and following mRNA analyses is hampered by technical and economical obstacles. Hence, improvements are urgently needed to identify causative synonymous and splicing variants.

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P08.21B

Somatic instability of CGG repeats in the *FMR1* gene is a factor in symptom severity in Fragile X syndrome patients

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Introduction: Fragile X syndrome is a main cause of inherited intellectual disability in humans. The molecular basis of the disorder is a CGG-repeat expansion in the *FMR1* gene. Normal repeat lengths are fewer than 50 triplets; the full mutation repeat size is more than 200 triplets. It has been shown that carriers of a large allele

exhibit repeat size somatic instability. This instability was suggested to be linked to symptom severity. Here, we present a study of the relationship between somatic instability in patients with changes in their brains and the severity of their psychiatric symptoms.

Materials and Methods: Measurements of the repeat size were carried out by fragment analysis on an ABI 3130XL Genetic Analyzer, and the MRIs were performed on an Achieva scanner with a magnetic field strength of 1.5 T. Patients were diagnosed by genetic and psychiatric assessments at NCCH No. 1.

Results: The efficiency of PCR in repeat sizing does not allow the calculation of the instability index by the quantitation of each allele, as has been done previously in mouse studies. We developed a novel approach for evaluating somatic instability in humans based on repeat sizes and the number of alleles. A strong correlation between somatic instability and changes in brain-region connectivity was found in Fragile X syndrome patients and in some brain regions of the patients' healthy mothers. Additionally, somatic instability significantly influenced the psychiatric and behavioral symptoms of Fragile X syndrome patients.

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P08.22C
PR*i*SM, a functional genomics screen to assess the pathogenicity of candidate variants of neurodevelopmental disorders

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Next generation sequencing (NGS) has become the method of choice to identify the presence of pathogenic mutations in children with a neurodevelopmental disorder. Recent studies suggested that a possible pathogenic genetic mutation can be identified in the majority of individuals with intellectual disability. However, many of the identified variants are genetic variants of unknown significance (VUS). To establish whether these variants are pathogenic, independent confirmation is needed. The PR*i*SM (Pipeline for Rapid *in silico/in vitro/in vivo* Screening of Mutations) is developed as a functional genomics screen to test the pathogenicity of such variants. (see: www.functionalgenomics.nl). Although we can make use of many specific biochemical read-outs to establish the pathogenicity of a variant (*eg*, kinase assays, ubiquitin assays) the core assays of the PR*i*SM screen are hypothesis-free: biological information about the protein is not a prerequisite to assess the pathogenicity of the variant. This allows us to assess whether variants in protein domains for which the precise function is unknown or cannot be assessed biochemically, are pathogenic. Moreover, we can test variants in proteins for which no biological information is available at all. During the presentation an overview will be given on the number of mutations tested until now using the PR*i*SM screening and their outcomes, discussing amongst others the sensitivity and specificity as well as the strengths and the weaknesses of this screen. Taken together, we have now setup a functional genomics screen which has already proven its value at the diagnostic as well as the scientific level.

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P08.23D
GATAD2B-Associated Neurodevelopmental Disorder (GAND), further delineation of the phenotype in a series of 10 patients

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Introduction: Pathogenic variants in the *GATAD2B* gene have recently been associated with a syndromic neurodevelopmental disorder (GAND) characterized by severe intellectual disability (ID), impaired speech, childhood hypotonia, and dysmorphic features. The majority of reported patients harbored *de novo* loss of function (LoF) variants. To date, the phenotype of only four patients has been precisely described in the literature, contrasting with the relative frequency of detection of such LoF *GATAD2B* variants. Here, we describe 10 patients with confirmed LoF *GATAD2B* variants and further delineate the clinical phenotype.

Methods: Patients were included by contacting referent clinicians from several French genetics departments.

Results: Developmental delay was severe with a median age of 2.4 years (range [2-5]) for independent walking and of 2.75 years (range [1-4]) for first spoken words. They showed very little subsequent progress, one patient remaining non-verbal at age 30 years. ID was mostly moderate, with only one severe and one mild case, which differs from the original description of severe ID. Most common dysmorphic features included broad forehead, deeply set eyes, hypertelorism, downturned mouth and, anomalies of the extremities. Conversely, prenatal complications, non-cerebral organ malformations, epilepsy and autistic behaviour were very rare. One patient presented asthma and recurrent respiratory infections, another one presented severe feeding difficulties and developed acute lymphoblastic leukemia and IgG deficiency.

Conclusions: NGS-based approaches for sequencing will improve the detection of *GATAD2B* variations. Better knowledge of the clinical phenotype is essential for a correct interpretation of the molecular results and for an accurate management of these patients.

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P08.24A

Mutations in *PIGU*, impairing the function of the GPI transamidase complex cause severe intellectual disability, epilepsy, and brain anomalies

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The glycosylphosphatidylinositol (GPI) anchor links over 150 proteins to the cell surface and is present on every cell type. Many of these proteins play crucial roles in neuronal development and function. Mutations in 18 of the 29 genes, implicated in the biosynthesis of the GPI anchor, have been identified as the cause of GPI biosynthesis deficiencies (GPIBDs) in humans associated with intellectual disability and seizures as the cardinal features. *PIGU is* an essential component of the GPI transamidase complex along with *PIGK*, *PIGS*, *PIGT*, and *GPAA1* that links GPI anchored proteins (GPI-APs) onto the GPI anchor in the ER. Here, we report two homozygous missense mutations (NM_080476.4:c.209T>A and c.1149C>A) in five individuals from three unrelated families. All individuals presented with global developmental delay, severe to profound intellectual disability, muscular hypotonia, seizures, brain anomalies, scoliosis, and mild facial dysmorphism. Using flow cytometry, a characteristic profile for GPI transamidase deficiency consisting of reduced cell surface expression of FLAER, CD16, and CD24, but not CD55 and CD59 on granulocytes and free GPI anchor detected by T5 antibody on B-cells, was determined. Moreover, computer-assisted facial analysis of different GPIBDs revealed a shared characteristic facial gestalt between individuals with mutations in *PIGU* and *GPAA1*. Our findings strengthen the role of the GPI transamidase complex in the development of nervous and skeletal systems and expand the clinical spectrum of disorders belonging to the group of inherited GPI anchor deficiencies.

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P08.25B

Cryptic unbalanced translocations causing intellectual disability or congenital malformations can be surprisingly large

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Unbalanced reciprocal translocations are a frequent cause of intellectual disability (ID) and are usually diagnosed by conventional cytogenetic diagnostics. However, some of them are only detectable by e.g. FISH, MLPA or chromosomal microarrays (CMA) and are thus cryptic.

Here, we present three unbalanced translocations detected after cytogenetic analyses with normal results at our institute in the last year. In Patient 1, a 10-year old girl with i.a. ID and dysmorphisms, CMA detected a 5.0Mb terminal gain of 2q and a 3.6Mb terminal loss of 21q. Surprisingly, the terminal imbalances in patients 2 and 3 were quite large: CMA detected an 11.4Mb loss of 10p and an 8.9Mb gain of 13q in Patient 2, a 10-month old boy with i.a. growth retardation, VSDs and choanal atresia. In Patient 3, a 15-year old boy with i.a. ID, short stature, microcephaly, seizures and renal agenesis, CMA detected a 10.4Mb loss of 9p and an 11.4Mb gain of 12p. FISH demonstrated unbalanced translocations in all patients and balanced maternal translocations for patients 1 and 2. For Patient 3, parental material was unavailable.

Especially patients 2 and 3 are instructive examples with imbalances greater than 10Mb which theoretically should be detectable by GTG-banding. However, the affected regions were very similar in size and banding patterns and thus escaped detection by banding methods. Our observations emphasize the importance of CMA not only in the detection of microdeletions and -duplications, but also of large chromosomal imbalances in patients with ID or congenital malformations.

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P08.26C

Exome sequencing of 100 patients with intellectual disability

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Introduction: Intellectual disability (ID) is a widespread group of diseases with a frequency of 1% to 3%. Next generation sequencing (NGS) technologies significantly reduced the time of diagnostics compared to single-gene sequencing. Optimal method of NGS is a whole (WES) or clinical (CES) exome sequencing.

Materials and Methods: The study included 100 patients with various forms of ID. All cases were submitted from geneticists. Martin-Bell, Rett syndromes and chromosomal pathology were excluded, the patients had no traumatic and infectious brain damage. WES was performed for 69 patients. CES (panel of 6500 HGMD genes) was performed for 31 patients. Founded gene variants were confirmed by Sanger sequencing in trios.

Results: We diagnosed syndromes (Kabuki, Cohen, Cowden, Nicolaides-baraitser, ZTTK, Smith-Magenis, Helsmoortel van der Aa, Cornelia de Lange-like, multiple congenital anomalies-hypotonia-seizures syndrome 1), metabolic diseases (MPS III type), early epilepsy encephalopathy (type 4 and 7), mental retardation autosomal dominant (7, 8, 35, 36, 49 types, ID with a mutation in the *GRIA1*), mental retardation X-linked (98 and 102 types), mesomelic dysplasia with a mutation in the *AFF3*. Several new candidate genes were identified. As a result, 21 pathogenic variants, 3 probably pathogenic and 12 variants of unknown significance were found.

Conclusions: The total diagnostic value of the exome sequencing is 24% (20% for WES; 29% for CES). Many nonspecific clinical signs in ID make it difficult to establish precise diagnosis without molecular genetic testing. In these cases, exome sequencing is a method of first choice.

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P08.27D
Whole exome sequencing in a cohort of adults patients with intellectual disability

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Intellectual disability (ID) is frequent (about 2% of the population, i.e. 1.3 million of persons). Etiologies are numerous and heterogeneous. We developed since 2005, as part of the Reference Center for ID of Rare Causes, a specific multidisciplinary consultation (neurologist, geneticist and social worker) for adults with ID which main objectives are diagnosis, genetic counseling, management, follow-up, phenotypic characterization, evolutionary profile and natural evolution of different genetic syndromes in adults. Many adults with ID have no diagnosis because they could not benefit from the latest high-throughput sequencing techniques. We focused on adults patients (older than 15 years) with isolated or syndromic ID without diagnosis. These patients were from pediatric departments (40%), adults' medical departments (30%) (whose neurological departments 20%) or referred from their general practitioner or practitioner of their institution (30%). 222 of these patients underwent high throughput sequencing in trios (patient and 2 parents) consisting in TruSight One gene panel (TS1, 51 trios, Illumina*) or whole exome sequencing (171 trios). We established a diagnosis in 21.6% of them with TS1 and 43% with WES. Whole exome sequencing diagnosis yield is comparable with series published in literature. In most of cases we found de novo mutations. We also found candidate genes needing validation with ongoing gene matching procedures. Identifying genetic causes is crucial for relatives' genetic counseling and to describe natural evolution of known syndromes with valuable information on evolutionary profile in adults.

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P08.28A
Involvement of FMN2 in nonsyndromic autosomal-dominant intellectual disability

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We report genetic analysis of a non-consanguineous Armenian family exhibiting extended history of non-syndromic intellectual disability (NSID) in four-generation pedigree, including affected six males (three sibs, father and two paternal uncles) and three females (daughter, paternal grand-mother and grand-grand-mother) with mild to moderate ID and with normal karyotype. Molecular karyotyping analysis of all affected relatives using Illumina HumanOmniExpress BeadChip identified only benign CNVs and no extended region of LOH. Assuming apparent autosomal dominant inheritance pattern of the clinic through four generations, all living affected six male and one female relatives were analysed by Whole Exome Sequencing analysis using Illumina´s SureSelect 62Mb Enrichment Kit and HiSeq2000 platform. Further variant calling and annotation by ANNOVAR revealed only one heterozygous genetic variation in all affected relatives: a putative exonic 33 bp non-frameshift deletion in FMN2 gene (NM_020066:c.2802_2834del) predicting a deletion of amino acids 934-945. The heterozygous variant was confirmed by Sanger sequencing in all affected relatives and was not detected in any of the healthy relatives (mother and sibs). FMN2 encodes a protein of the formin family of actin cytoskeleton nucleation factors which is highly expressed in the maturing brain. FMN2 as a candidate gene for ID had been speculated in reports of two sporadic cases with interstitial deletions involving FMN2 and suggesting the mechanism of haploinsufficiency which was contradicted to the further report of homozygous truncating mutations in FMN2 as cause of autosomal-recessive NSID in two consanguineous families. This is the first report of FMN2 intragenic mutation causing autosomal-dominant form of NSID.

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P08.30C
Telling the untold story: The economic and psychosocial impacts on families affected by intellectual disability

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Introduction: Data on the financial and psychosocial costs incurred by families affected by intellectual disability (ID) is scarce, thus limiting the capacity to value the benefits of diagnosing and preventing ID through the application of genomic testing. We report on the first large, in-depth study exploring the economic, psychosocial and potential reproductive impacts of whole genome sequencing (WGS) for ID.

Materials and Methods: A survey instrument was developed to assess quality of life, psychosocial impacts, education, employment, income, wealth, welfare dependency, living arrangements and family out-of-pocket costs. Eligible families had WGS. Data on 175 individuals with ID (100 households) has been collected.

Results: Preliminary data showed combined costs to the Australian and State Governments, and private households totalled $13.1million per household up to the age of 69. Families bore a significant financial burden ($4.8million per household) mainly due to lost income and out-of-pocket expenses. Families were under enormous psychosocial strain and most carers reported having a poor quality of life. Costs to Governments were $10.7million per household, with the main costs being for special education, residential care and welfare. Among the families who have had WGS results returned, 44% have had a diagnosis with a further 12% having a potential diagnosis. Most families would consider using assistive technologies such as prenatal diagnosis and IVF with preimplantation genetic diagnosis in future pregnancies.

Conclusions: Families affected by ID experience a significant financial and psychosocial burden. This data is important for benchmarking the potential benefits of WGS in familial ID.

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P08.31D
The benefits of whole exome sequencing data reanalysis in Intellectual disability

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Nowadays, whole exome sequencing has become a widespread tool in studying Mendelian disorders. The diagnostic yield is assumed around 25-40% and the negative cases are due to WES limitations. Besides the technical issues such as low coverage in specific regions or random failure in hybridization reaction, inaccuracies in different data analysis steps play a significant role. These can be resolved by updates in bioinformatics tools, algorithms, databases and literature, improving the diagnostic yield of at least 10-20% in different studies. Recently, we have published the results of a large cohort of patients presenting intellectual disability in which the diagnostic yield of 54% was achieved. In present study, 172 negative cases from previous cohort were selected aiming to improve diagnostic yield by re-sequencing and/or re-analysis. So far, the analysis of 68 patients is accomplished of which, the possible causative variant is detected in 12%. These results are ascertained through three main strategies. First, extensive clinical follow up, re-sampling and re-interpretation of previous WES data led to identification of one known and two novel candidate genes; *ASPM*, *TTI1* and *GPR126*. Second; WES-reanalysis applying improved algorithms and filtering strategies in addition to updates of disease-causing genes revealed two possible candidates; *AKTIP*, *MAZ*, and two known genes; *CDK10*, *FSCN1*. Third; re-sequencing revealed another candidate gene; *CCDC58* possibly by expanding the coverage. In conclusion, this project has shown 12% improve in diagnostic yield so far, also introduced five possible novel candidate genes and provided a second report for the recently identified genes; *CDK10* and *FSCN1*.

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P08.32A
Mutation in *C1orf131*, encoding a novel nucleolar protein, causes intellectual disability in a large Pakistani family

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Introduction: Intellectual disability (ID) is characterized by substantial limitations in both intellectual functioning and adaptive behavior. 50% of ID cases have genetic basis. It can also be categorized as syndromic intellectual disability (S-ID) and non-syndromic intellectual disability (NS-ID). Worldwide prevalence of this disorder is estimated to range from 1% to 3%.

Material and Methods: For genomic analyses, homozygosity mapping was coupled with whole-exome sequencing (WES) whereas immunofluorescence, confocal microscopy, pulldown assays and mass spectrometry analyses were performed for biochemical analyses.

Results: We studied a Pakistani family of NS-ID and identified linkage regions with a maximum possible LOD score of 2.4 at chromosomes 1, 2, 15 and 21. WES conducted on DNA of two affected members revealed a missense mutation (NM_152379.3:c.112G>A,p. Asp38Asn) in *C1orf131*, encoding the uncharacterized protein C1orf131, as a likely cause of ID. The mutation replaces a highly conserved aspartic acid by asparagine and is predicted to be pathogenic by Mutation Taster and Polyphen-2. We show here that C1orf131 is a novel nucleolar component and relocates to the chromosomal periphery during mitosis. Mutant primary fibroblasts exhibit reduced and distorted nucleoli, micronuclei and misshapen nuclei. Similar effects are seen upon overexpression of mutant protein or knockdown by siRNA. Our data also show that C1orf131 interacts with several nucleolar and centrosomal proteins.

Conclusion: We conclude that *C1orf131* is a novel gene associated with ID and that its protein product plays a crucial role in maintaining the structural integrity of the nucleolus. It is also an essential component for normal brain functions.

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P08.33B

Genetic diagnosis of intellectual disability: results of trio-based whole exome sequencing in a cohort of 818 patients

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Introduction: Intellectual disability (ID) is a clinically and genetically heterogeneous condition affecting 1 to 3% of population worldwide. Recent improvements of new generation sequencing are transforming medical practice. We present the clinical and genetical characteristics of a large cohort of patients suffering from ID and had a trio-based whole exome sequencing (WES).

Patients and Methods: 818 patients were recruited through clinical genetic and/or neuro-pediatric consultations. ID was assessed based on neuropsychological tests when available, DSM-5 classification was used otherwise. Prior to WES, a DNA micro-array, a search for Fragile-X syndrome and a targeted-gene analysis in case of high syndromic suspicion were performed. If negative, trio-based WES was proposed. Molecular results were discussed in multidisciplinary team meetings before feed-back to patient.

Results: The global diagnostic yield was 42% across all patients (338/818 patients). 41 mutations were found in candidate genes and contributed to their validation as "ID genes", rising the potential diagnostic yield to 47% (379/818 patients). Dominant mutations were pre-eminent (81%) and most of them were *de novo* (95%) affecting 224 unique genes. Interestingly, severity of ID had no statistically significant effect on diagnostic rate (mild ID, 66/180, 37% - moderate to profound ID, 108/419, 44%, p-value = 0.1997). Epilepsy and family history of ID were not identified neither as cofounding factors (p-value = 0.132 and 0.516).

Conclusion: This study emphasizes the considerable genetic heterogeneity of ID and confirms the efficiency of trio-based WES for its exploration and delineation in a genotype-first approach.

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P08.34C

Haploinsufficiency of PHF21A due to frameshift and nonsense mutations causes syndromic autism including intellectual disability, craniofacial anomalies, epilepsy, hypotonia, and neurobehavioral problems

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PHF21A encodes a protein that specifically binds unmethylated H3K4 as part of a histone demethylase complex that participates in suppression of neuronal gene expression. It had been associated with intellectual disability and craniofacial anomalies, based on its deletion in Potocki-Shaffer syndrome (PSS) interval at 11p11.2 and its disruption in three patients with balanced translocations. However, until now, individuals who carry deleterious sequence variants within *PHF21A* have not been identified. Moreover, the identity of a novel gene underlying hypotonia or neurobehavioral phenotype at 11p11.2 remained cryptic. Here, we describe seven individuals who carry heterozygous intragenic sequence variants in *PHF21A:* a missense variant, which alters the last nucleotide of an exon, four frameshift variants, and two identical nonsense variants. These variants are predicted to trigger nonsense mediated mRNA decay (NMD) or generate a truncated version of PHF21A. This confirms that haploinsufficiency of *PHF21A* causes not only intellectual disability and craniofacial anomalies, but also epilepsy, language delay, hypotonia, and neurobehavioral problems. We also found that PHF21A is profoundly expressed in human fetal brain and skeletal muscle. This emphasizes the role of PHF21A in early human development, which is consistent with developmental delay, autism, ADHD, epilepsy, and hypotonia observed at an early age in our patients. These findings definitely provide proof of the pathogenicity of this gene in a syndromic form of intellectual disability. Furthermore, our studies suggest additional features, in particular autism, which extend the phenotypic spectrum attributable to *PHF21A* mutations.

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P08.35D

RNFT2*,a novel gene causing intellectual disability; functional evidence in *Drosophila melanogaster

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Introduction: Intellectual disability (ID) as the most prevalent disability in the world and a heterogeneous disease is one of the major unsolved issues in health care. The advent of next generation sequencing (NGS) technology has resulted in an exponential increase in deciphering of novel ID-causing genes. Whole exome sequencing (WES) in a part of large cohort study on 404 Iranian consanguineous families with autosomal recessive intellectual disability (ARID) led to the identification of a novel likely pathogenic missense variant (cT1150C; p.C384R) in *RNFT2* gene. This variant is located in highly conserved zinc finger domain that may change the protein structure in a way to reduce the $zinc^{2+}$ affinity to protein active site and consequently leading to possible alteration in the DNA binding site. To provide functional evidence for the effect of this gene on cognition, we used transgenic *Drosophila* model.

Material and Methods: Using Gal4/UAS genetic tool *RNFT2*-ortholog gene (CG13605) in *Drosophila*

melanogaster was down-regulated in the fly brain by RNAi mechanism. Then the impact of this gene manipulation on learning ability and short-term memory of the flies were studied using olfactory conditioning assay.

Results: Remarkable decrease in olfactory memory performance indices were observed in the flies underwent CG13605 down regulation when compared to control genotype ($P < 0.05$).

Conclusion: Our finding suggested the possible role for *RNFT2* in functionality of *Drosophila* brain in terms of learning and short-term memory which support its probable role in inducing human cognitive impairment.

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P08.36A

Biallelic variants in *IQSEC1* cause intellectual disability, developmental delay and short stature

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We analyzed two consanguineous families with intellectual disability in which homozygous missense likely pathogenic variants were identified in the *IQSEC1* (NM_001134382.3) gene. In the first family with two affecteds from Pakistan, the *IQSEC1* missense segregating variant was (c.1028C>T: p.(Thr343Met)) while in the second family of three affecteds from Saudi Arabia, the missense variant was (c.962G>A:p.(Arg321Gln)). Five affected individuals from the two families manifested a similar phenotype that includes intellectual disability, developmental delay, short stature, aphasia/speech problems, and hypotonia. Affected individuals with the Arg321Gln biallelic variant also have early onset epilepsy. The X-linked paralog IQSEC2 has previously been linked with intellectual disability and epilepsy. Mice with a conditional *Iqsec1* deletion in cortical neurons exhibited an increased density of dendritic spines with an immature morphology. The Drosophila ortholog of *IQSEC1* is schizo(siz). *Siz* is broadly expressed in CNS and PNS from embryo to adult. RNAi knockdown of *siz* in neurons does not affect viability, whereas glial specific knockdown exhibits severe pupal lethality, suggesting that siz has a critical role in glia rather than neurons. The identified *IQSEC1* missense variants behave as severe loss-of-function alleles in fly given that ubiquitous expression of human IQSEC1 is toxic to the fly so that we failed to obtain viable flies, whereas expression of the two missense variants consistently produced viable animals. The phenotypic similarity of the patients in the two families, and the functional experiments in mice and Drosophila suggest that *IQSEC1* pathogenic variants cause intellectual disability, developmental delay and shot stature, in the autosomal recessive manner.

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P08.37B

Deletion of *KCNK9*, an imprinted gene on 8q24.3 with maternal expression, is associated with dysmorphic features and developmental delays

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Introduction: *KCNK9* imprinting (Birk-Barel) syndrome (MIM 612292) is a rare syndrome characterized by congenital hypotonia, dysmorphic features, developmental delay and intellectual disability. It is caused by pathogenic variants in *KCNK9*, which is an imprinted gene located on 8q24.3 and is normally expressed on the maternally inherited chromosome. To date, the small number of reported cases of *KCNK9* imprinting syndrome have been attributed to 3 missense variants. There is no known phenotype associated with a deletion of *KCNK9*.

Methods and Results: We describe the case of a 5 year old male who presented at age 3 with global developmental delay and dysmorphic features including prominent supraorbital ridges and a prominent nasal root. A chromosomal microarray revealed a 0.014 Mb deletion on 8q24.3 of uncertain significance, encompassing 1 OMIM gene, *KCNK9*. This deletion was maternally inherited. Notably, the proband's mother had similar dysmorphic features and intellectual disability. It was determined by SNP microarray that the proband's mother herself had a *de novo* maternal deletion on 8q24.3.

Conclusions: This is the first report of a clinically affected mother and child with deletion of the imprinted region 8q24.3, encompassing the gene *KCNK9*. Further studies will contribute to our understanding of the mechanism and spectrum of *KCNK9* associated disorders.

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P08.39D
MED12 missense mutation in a three-generation family: further evidences for a fourth distinct phenotype

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Mutations in *MED12* gene have been described in association with syndromic et non-syndromic X-linked intellectual disability (XLID). Up to date at least three distinct XLID syndromes have been described: FG syndrome, Lujan-Fryns syndrome (LS) and Ohdo syndrome (OSMKB). In the last years, thanks to the massive use of next generation sequencing techniques (NGS) it has been possible to discover at least 13 others *MED12* mutations and to expand the phenotype of *MED12*-related disorders. Here we report three subjects from a large non-consanguineous family presenting with a mild to severe ID, important speech delay, behavior problems, dysmorphic facial features and hearing loss. NGS allows us to detect the *MED12* missense variant c.3883C>T (p.R1295C) carried by the three patients. This variant has been reported in 2016 by Hu *et al* in one family from a big cohort of XLID unresolved families. The clinical and phenotypic variability between the patients described by the different authors through the last years is very wide, and it could be explained by the multiple functions of the MED12 protein in complex pathways. This clinical report contributes to expanding the phenotype associated with *MED12* mutations.

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P08.40A
Functional delineation of de novo heterozygous intragenic deletion in *MED13L*

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Introduction: Heterozygous pathogenic variants in *MED13L* (MIM #608771) gene cause intellectual disability and distinctive facial features with or without cardiac defects (MIM #616789). This complex neurodevelopmental disorder is characterized by various phenotypic features including plagiocephaly, strabismus, clubfoot, poor speech, and developmental delay, which are present in our proband.

Materials and Methods: SNP-CGH followed by functional genome analysis was conducted for the proband. In order to elucidate the pathogenicity of the intragenic *MED13L* gene deletion, proband's total blood RNA was isolated and cDNA was synthesized. Subsequently, a quantitative RT-PCR using TaqMan gene expression assays designed for *MED13L* gene was performed. Lastly, *MED13L* gene was sequenced in patient's cDNA sample by Sanger method.

Results: A *de novo* 97.88 kb deletion in the cytoband 12q24.21 including 3rd and 4th exons of *MED13L* gene was detected. Further quantitative expression analysis of several *MED13L* exons disclosed the deletion of exons 3-4 but excluded the deletion of exons 1-2 and 16-17. The borders of the deletion in the coding region were narrowed by Sanger sequencing. The deletion spanning from the exon 3rd to 9th was determined. *In silico*, the deletion was predicted to result in truncated protein NP_056150:p.(Val104-Glyfs*5) partly altering Med13_N domain and losing medPIWI and Med13_C domains.

Conclusions: Based on these findings, heterozygous deletion results in partial haploinsufficiency of the *MED13L* gene in the affected patient due to the premature termination of the protein translation.

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P08.41B
The clinical phenotype of *ANKLE2*-related microcephaly in 4 families

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Few patients with autosomal recessive *ANKLE2*-related congenital microcephaly have been described since Yamamoto's report in 2014. We describe four families (6 children) with *ANKLE2*-related microcephaly, including the boy from the original report (Family 1). In Family 1, two Hispanic siblings have *ANKLE2* variants: c.2344C>T (p.Q782X); c.1717C>G (p.L573V). The male had low birth weight (BW), HC -5.9 SD, sloped forehead, scalp rugae, and hyper- and hypopigmented macules (HHPM). At age 13, he has spastic quadriparesis, partial complex seizures, short stature, and severe intellectual disability without speech. He is fed by gastrostomy tube. His microcephalic sister with HHPM, died hours after birth. In Family 2, two microcephalic Iraqi sisters, with consanguineous parents, have a homozygous *ANKLE2* variant: c.686T>G (p.V229G). They have HC -8-10 SD, scalp rugae, short stature (-2-3 SD), and HHPM. Both walk and talk with severe neurodevelopmental delay. In Family 3, a Northern European girl with HC -6 SD, without scalp rugae or pigmentation, has two *ANKLE2* variants: c.325G>C (p.A109P); c.1421-1G>C. She walks & talks with mild-moderate neurodevelopmental delay. In Family 4, an Indian/Filipino girl has low birth weight (-7.08 SD), microcephaly (HC -7.08 SD), HHPM and two *ANKLE2* variants: c.706C>T (p.R236*); c.1606C>T (p.R536C). She has a closed anterior fontanel and normal neurologic exam at 2 weeks. All affected children have severe microcephaly, simplified cortical gyri and variable developmental delay. Frontal lobe foreshortening, corpus callosal dysgenesis, scalp rugae, and HHPM are common but not universal. The triad of severe congenital microcephaly, scalp rugae and HHPM suggests this diagnosis.

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P08.42C
Neurochondrin missense variant associated with autosomal recessive intellectual disability and epilepsy

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Introduction: The *NCDN* gene encodes neurochondrin, predominantly expressed in neural tissues where it mediates neural outgrowth, synaptic plasticity and signal transduction. Neurochondrin acts as an endogenous modulator of glutamate receptor mGluR5 and in mice, depletion of *Ncdn* causes epileptic seizures, depressive-like behaviors and impaired spatial learning.

Material and Methods: We identified a consanguineous Pakistani family segregating mild intellectual disability and seizures in three children. Whole-exome sequencing was performed on samples from two affected siblings. Human neuroblastoma line SH-SY5Y and CRISPR/Cas9 editing was used to model loss of *NCDN in vitro*.

Results: We identified a rare homozygous *NCDN* missense variant (NM_001014841.1:c.1246G>C) in the three affected family members. Electroencephalography (EEG) of two affected siblings revealed epileptic pattern whereas brain MRI was normal. Using CRISPR/Cas9, we then generated *NCDN* knockout (KO) in SH-SY5Y cells. The KO cells exhibited significantly reduced neurite outgrowth as compared to w.t. cells. Neurochondrin modulates the activation of mGluR5 of importance for ERK1/2-phosphorylation and we then examined the phosphorylation of ERK1/2 at position threonine 202. The ERK1/2-phosphorylation was reduced in *NCDN* KO cells when compared to w.t. SH-SY5Y cells. Conversely, phosphorylation increased when *NCDN* was overexpressed in both w.t. and KO SH-SY5Y cells.

Conclusion: *NCDN* loss of function in SH-SY5Y cells revealed impaired neurite outgrowth and reduced mGlu5R dependent ERK1/2-phosphorylation. Our data support a role for neurochondrin in neurodevelopment and show that a *NCDN* missense variant is associated with autosomal recessive intellectual disability and epilepsy. This study was supported by Swedish Research Council (2015-02424) and Hjärnfonden (FO2018-0100) to ND.

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P08.43D

Genotype-Phenotype correlation of 11 new Spanish patients with Bainbridge Ropers syndrome

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Introduction: Bainbridge-ropers syndrome (BRPS) is a neurodevelopmental disorder caused by mutations in *ASXL3*. Patients with BRPS show delayed psychomotor development, intellectual disability, speech alterations, hypotonia, poor growth and dysmorphic features. It is categorized as a syndromic cause of Autism spectrum disorder (ASD). Nowadays, 33 patients with BRPS have been reported. Our work expands the mutational spectrum of the *ASXL3* with the genotype-phenotype characterizing 11 new patients.

Material and Methods: In a collaborative initiative we have identified 11 patients with neurodevelopmental disorders and ASD phenotype with loss of function(LoF) mutations in the *ASXL3* gene. Exome sequencing was used as a diagnostic tool and Sanger sequencing was used for familial segregation. The mutational spectrum of *ASXL3* was revised in public_data (PubMed, Clinvar, SFARI).

Results: We identified 10 new *ASXL3* LoF variants and one recurrent mutation (c.3106C>T;p.Arg1036Ter). The main clinical characteristics of our patients included moderate-severe intellectual disability with ASD traits and neuroconductual disorder including aggressively behavior in adolescence. Dysmorphic features highly suggestive of *ASXL3* were delineated eyebrows and the skeletal phenotype. So far, 77 different LoF mutations (48 frameshifts, 28 Stop Gain, 1 splicing) have been identified including our present results. There are 3 recurrent mutations and 73 unique variants.

Conclusions: The inclusion of new patients with BRPS increases the knowledge of mutational spectrum of *ASXL3* and is essential to further delineate the phenotype of this syndrome. The recognition of this syndrome in the context of a neurodevelopmental disorder is indispensable for the orientation of molecular diagnosis.

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P08.44A

Neurodevelopmental disorders: a next generation

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Since 2010 whole exome sequencing (WES) discovered a large numbers of novel genes and neurodevelopmental disorders (NDDs), denoted here as 'next-generation NDDs'. Yet, ~40% of NDD patients remain undiagnosed. Therefore, we previously established a cohort of 826 patients with unexplained ID, a subtype of NDD, for whom trio-based WES was performed. ID severity ranged from mild (27%) to severe (27%) and 32.2% of patients had other NDD-subtypes. We identified pathogenic variants in known NDD genes in 28.6% of patients and 386 patients (46.7%) had at least one *de novo* mutation in a gene not previously associated with NDDs. Between 2014 and 2018, we have so far confirmed seven of 586 genes as next-generation NDD-gene (*SON*,*TLK2*,*RAC1*,*RHEB*,*USP9X*,*RAB11B*,*WAC*). Matchmaking was successful for all but one (*RHEB*), resulting in a maximum of 35 additional patients (*TLK2*) and underscoring the need for data-sharing for next-generation NDDs. Additionally, mutations in these genes together only diagnosed ~1% of patients of our initial cohort, illustrating the rarity of next-generation NDDs. Of note, for *USP9X*, *WAC* and *TLK2*, most patients were mildly affected. Further systematic analysis of our cohort showed that overall a similar percentage of *de novo* mutations in known NDD genes was present in patients with mild ID (27.9%) compared to more severe ID (27.2%), indicating that WES should not be restricted to patients with more severe phenotypes. Finally, we observed a challenge to translate the discovered next-generation NDDs to clinic. To improve patient care, we propose an organizational model, in which parents have a key role.

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P08.45B
Genetic Interaction screen for severe neurodevelopmental disorders reveals a functional link between *Ube3a* and *Mef2* in *Drosophila melanogaster*

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Neurodevelopmental disorders (NDDs) are clinically and genetically extremely heterogeneous. Shared phenotypes are often caused by mutations in genes involved in the same networks, complexes or pathways. Mutations in *TCF4, MEF2C, UBE3A, ZEB2* or *ATRX* cause overlapping, syndromic forms of severe NDDs with epilepsy, thus suggesting molecular and functional commonalities.

Transcriptome analysis on RNA from patients' peripheral blood (mutations in either *TCF4*, *MEF2C*, *ZEB2* or *ATRX*) revealed common transcriptional targets as pairwise comparisons of deregulated genes showed significant overlap. Among these we found enrichment for GO terms such as RNA-related and ubiquitin ligase-related processes and significant enrichment of known NDD associated genes, indicating central roles in neurodevelopment.

Next, we screened for genetic interactions in *Drosophila melanogaster* by inducing ubiquitous or tissue specific knockdown or overexpression of each single gene and in pairwise combinations. We assessed parameters such as lethality, wing and eye morphology, neuromuscular junction morphology and bang sensitivity and climbing behaviour. We observed robust genetic interaction between *Ube3a* and *Mef2* by multiple phenotype modifications upon simultaneous dosage manipulation in different tissues including glia, wing and eye. E.g. single overexpression of either *Ube3a* or *Mef2* resulted in mild eye phenotypes (rough surface or reduced bristles), respectively, while combined overexpression led to a severe eye size reduction and disorganized ommatidia structure, indicating synergistic interaction.

Assessment of genes implicated in clinically overlapping NDDs revealed commonly deregulated target genes and genetic interactions between *Ube3a* and *Mef2* in the *Drosophila* model system, suggesting that such perturbed interactions contribute to the underlying pathomechanisms also in humans.

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P08.46C
Exome Sequencing and functional follow up reveal MAB21L1 and PPP1R21 loss of function causes distinctive syndromal neurodevelopmental phenotypes

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Introduction: Next-generation sequencing has been instrumental in solving the genetic basis of rare inherited diseases, especially neurodevelopmental syndromes. A large number of novel genes has been identified in the last

years, however those account for only 50%-70% of all cases. Further, functional workup is essential for precise phenotype definition and to understand the underlying disease mechanisms.

Materials and Methods: Using exome sequencing (ES), we have analysed a cohort of 100 Iranian individuals affected by developmental delay/ intellectual disability.

Results: While 49% carried causative mutations in genes previously associated with neurodevelopmental disorders in human, we identified novel candidate genes in 29%. 22% remained unsolved. Amongst novel genes, we identified *MAB21L1* loss of function resulting in cerebellar, ocular, facial and genital features (COFG syndrome) and *PPP1R21* loss of function causing hypotonia, neurodevelopmental delay, facial dysmorphism, eye sight loss, loss of white matter and thinning of the corpus callosum as well as hepatosplenomegaly in some cases. We further established that PPP1R21 was absent in fibroblasts of an affected individual and consistent with the subcellular expression pattern and the clinical phenotype exhibiting features of storage diseases, we found patient fibroblasts exhibited a delay in clearance of transferrin-488 while uptake was normal. This suggests a role of PPP1R21 within the endosomal sorting process or endosome maturation pathway.

Conclusions: Our data demonstrates ES is an efficient diagnostic method for neurodevelopmental phenotypes. In addition, we delineate novel neurodevelopmental syndromes and show that bi-allelic *PPP1R21* loss of function variants affect the endosomal sorting process or endosome maturation pathway.

M. Najafi: None.

P08.47D

NONO-related syndromic intellectual disability: five new patients and carrier mothers with novel features

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Introduction: Pathogenic variants in the X-linked *NONO* gene are associated with intellectual disability, abnormal corpus callosum, left ventricular non-compaction, slender build, macrocephaly and dysmorphism in males. Only seven patients have previously been reported. Here, we present five new male patients with pathogenic variants in *NONO*, with several novel features.

Methods: All patients were ascertained after routine referral to their local Clinical Genetics service. Samples from three individuals underwent exome sequencing as part of the Wellcome Trust Deciphering Developmental Disorders study. One patient had whole genome sequencing performed as part of the 100,000 Genomes Project, and one had service trio exome analysis.

Results: Four patients had truncating pathogenic variants in *NONO*, and one splice site. All were maternally inherited. All patients had significant developmental delay and hypotonia. Three required gastrostomy tube placement, which has been reported in only two patients previously. Two had relative macrocephaly. None of the patients in our series have left ventricular non-compaction. All had abnormal cranial MRI scans. Novel features include periventricular leukomalacia, mild aortic root dilatation, and Ebstein anomaly. Recognizable shared dysmorphic features include frontal bossing, infra-orbital grooves and long fingers.

Discussion: Our series further expands and delineates the phenotypic spectrum associated with variants in *NONO*. This should prove useful to Geneticists, particularly in interpretation of genomic data.

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P08.49B

Improved delineation of the clinical spectrum and identification of previously unreported respiratory problems in a large participatory cohort study of Koolen-deVries syndrome through the GENIDA project

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Introduction: About 50 recurrent CNVs and 800 genes are implicated in genetic forms of intellectual disability (ID) with or without autism (ASD). The extreme heterogeneity of these neurodevelopmental disorders and the rarity of identified cases for many of the implicated genes renders the goal of defining the clinical spectrum, comorbidities and natural history a daunting task. We reasoned that a participatory approach would be a relevant alternative and used the Koolen-deVries syndrome (KdvS - 17q21.31del / KANSL1mut) for a proof-of-concept.

Methods and Results: We initiated GENIDA, a participatory online cohort study for specific genetic causes of ID/ASD (https://genida.unistra.fr), whereby clinical information is entered and updated by the family of the proband using a structured online questionnaire currently available in 5 languages, with answers from 650 participating families. The KdVS cohort is the largest, with 179 participating families. Results obtained for the frequency of behavioral problems, epilepsy or other previously reported comorbidities are consistent with published data. Our approach allowed us to refine frequencies of specific manifestations and their perceived severity. Inclusion of probands from a wide age range generates data on natural history. A major finding is the identification of recurrent respiratory problems in 40% of patients, including asthma (30 cases) and pneumonia (17 cases, mostly before 10y) that appear uncorrelated with reported laryngo/tracheomalacia or immunologic problems.

Conclusion: Data comparisons show that parents of patients can adequately answer the questionnaire, and validate our participatory approach. The finding of novel and relevant comorbidities has now to be translated in improved healthcare.

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P08.50C

Novel *de novo* mutations in *PHF6:* two unrelated females with Borjeson-Forssman-Lehmann syndrome

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We report two unrelated female patients with variable cognitive impairment and distinctive facial appearance. The first patient showed severe developmental delay, thin corpus callosum (CC), prominent supraorbital ridges with synophrys, deep set eyes and fleshy earlobes: in order to validate our clinical suspicion of Borjeson-Forssman-Lehmann syndrome (BFLS), we performed *PHF6* mutational analysis that revealed a *de novo* missense mutation within the PHD-type 2 domain; X-inactivation analysis showed skewed X-inactivation in blood lymphocytes. The second patient had severe delayed language development, intellectual disability (ID), hypermetropia, strabismus, linear skin hyperpigmentation and increasing obesity: PWS-AS test and array-CGH resulted normal. Whole-exome sequencing (WES), surprisingly, disclosed a *de novo* missense mutation in *PHF6*, in the same functional domain as our first patient; in this case, X-inactivation analysis showed normal X-inactivation in blood lymphocytes. BFLS is an X-linked recessive disease, characterized by ID, epilepsy, hypogonadism, hypometabolism, obesity and a typical facial gestalt; female carriers usually not show any findings or present only mild symptoms. To date, *de novo* aberrations in *PHF6* were reported in 13 females with a variable phenotype, characterized by ID, characteristic facial features, fingers and dental anomalies, only partially overlapping with BFSL as described in males. Molecular genetics has opened a new path to understand the complexity of ID, mostly in female patients, where we would tend to exclude a priori a known as X-linked recessive condition. However, in cases with heterogeneous ID phenotypes, WES is the instrument to address the exact diagnosis, as suggested in our second case.

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P08.51D

Elucidation of the phenotypic spectrum and genetic landscape in primary and secondary microcephaly

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Introduction: Microcephaly is a sign of many genetic conditions but has been rarely systematically evaluated. We therefore comprehensively studied the clinical and genetic landscape of an unselected cohort of patients with microcephaly.

Materials and Methods: We performed clinical assessment, high-resolution chromosomal microarray analysis, exome sequencing and functional studies in 62 patients (58% with primary microcephaly (PM), 27% with secondary microcephaly (SM), and 15% of unknown onset).

Results: We found severity of developmental delay/intellectual disability correlating with severity of microcephaly in PM, but not SM. We detected causative variants in 48.4% of patients and found divergent inheritance and variant pattern for PM (mainly recessive and likely gene-disrupting (LGD)) versus SM (all dominant de novo and evenly LGD or missense). While centrosome-related pathways were solely identified in PM, transcriptional regulation was the most frequently affected pathway in both SM and PM. Unexpectedly, we found causative variants in different mitochondria-related genes accounting for ~5% of patients, which emphasizes their role even in syndromic PM. Additionally, we delineated novel candidate genes involved in centrosome-related pathway (SPAG5, TEDC1), Wnt signaling (VPS26A, ZNRF3) and RNA trafficking (DDX1).

Conclusions: Our findings enable improved evaluation and genetic counseling of PM and SM patients and further elucidate microcephaly pathways.

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P08.52A

New patient with *de novo* nonsense *PRR12* variant supports role of this gene in neurodevelopmental disorder and helps to define phenotypic spectrum associated with *PRR12* haploinsufficiency

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The *PRR12* gene (19q13.33) encodes a large (2036 residues) proline-rich nuclear protein suspected to participate in neural development. The protein interacts with FMRP and the gene has high constraint to loss-of-function variation (gnomAD: 0 variants observed / 58 expected). Three *de novo* loss-of-function *PRR12* variants and one translocation disrupting the gene have been reported in the literature in patients showing global developmental delay, intellectual disability, neuropsychiatric problems, eye and vision abnormalities, dysmorphic features, hypotonia, skeletal abnormalities, sleep problems and anxiety. Three other *de novo PRR12* variants have been identified in large studies in additional patients from whom no or only limited phenotype information is available.

We describe a 3-year-old boy referred for developmental delay, failure to thrive and congenital hypotonia. His *facial features* included short forehead, bilateral epicanthal folds, hooded upper eyelids, malar hypoplasia, and borderline small asymmetric low-set ears. He also showed anxiety and autistic traits, and had pes planus and tapered digits. Exome sequencing revealed a *de novo* heterozygous *PRR12*

nonsense variant NM_020719.2(PRR12):c.3958C>T, p.(Arg1320*) which was absent from all databases. The facial phenotype was consistent with previously reported cases, except that no significant abnormalities in structure and function of the eyes were present.

Our findings support the role of *PRR12* haploinsufficiency in a rare neurodevelopmental disorder, and show, in accord with several other cases, that the eye phenotype originally described as a part of the typical presentation may be absent. Additional patients will further specify the phenotype associated with *de novo PRR12* variants.

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P08.53B

Genotype first approach in four patients with three rare autosomal recessive neurodevelopmental disorders

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Neurodevelopmental disorders are a heterogeneous group of very rare conditions which make up a significant part of agenda of medical geneticists. Targeted diagnostic methods have a limited chance to reveal their aetiology. Global "genotype first" approaches such as exome sequencing (ES) have a better diagnostic yield. However, their results and the genotype-phenotype correlation must be thoroughly revised to prevent misdiagnosis due to the lack of experience with rare conditions. We present four patients with three such conditions diagnosed by ES. In all cases the neurological impairment was dominant, and dysmorphic and other features were unspecific or absent. Patient 1 was a girl with progressive spastic tetraplegia, dysarthria and intellectual disability (ID). ES identified variants p.(Trp1253*) and p.(Lys849*) in *ALS2*. Phenotype revision supported the diagnosis of juvenile primary lateral sclerosis (MIM205100). Patient 2 was a girl with short stature, microcephaly, moderate ID, spastic paraplegia, aggressive behaviour and convergent strabismus. ES revealed variants p.(Pro648Ser) and p.(Ile403Thrfs*80) in *ALDH18A1*. Her phenotype corresponded to autosomal recessive spastic paraplegia type 9B (MIM616586). Patients 3 and 4, a sister and brother with similar neurological impairment, showed hypotonia and moderate ID. ES revealed variants p.(Ser893Arg) and p.(Leu311Trp) in *PIGN*, associated with multiple congenital anomalies-hypotonia-seizures syndrome type 1 (MIM614080). All three conditions belong to very rare diseases unlikely to be diagnosed solely based on clinical evaluation and targeted testing. Careful evaluation of variants identified using ES, detailed phenotype re-evaluation and thorough genotype-phenotype correlation is needed to prevent misinterpretation of findings in such cases. Supported by 17-29423A and 00064203.

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P08.54C

***SETD1A Loss of function* causes a distinct neurodevelopmental disorder in human and impaired memory in *Drosophila* further highlighting a conserved role of H3K4 methylation in brain function**

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Defects in histone methyltransferases (HMTs) are a major contributing factor to Neurodevelopmental disorders (NDDs). Loss-of-function (LoF) variants of *SETD1A* (*KMT2F*), one of the genes involved in histone 3 Lysine 4 (H3K4) methylation, have been identified in several individuals from large schizophrenia cohort studies. Interestingly, dominant gene mutations were also identified in children with developmental delay. To provide further insight in the somatic and behaviour profile, we further characterized the *SETD1A* associated Mendelian syndrome by collecting the molecular and clinical features of 15 so far unpublished individuals with *SETD1A* mutations via a GeneMatcher collaboration. Furthermore, to gain insight

into the role of SETD1A in fundamental learning and memory processes, we studied a Drosophila knock down of the orthologue Set1.

The cohort mainly comprised de novo variants that predict a loss of function with c.4582-2_4582delAG being a recurrent mutation resulting in intron retention. The predicted LoF of *SETD1A* leads to a distinct set of symptoms comprising global developmental or intellectual disability, subtle facial features, behaviour and psychiatric problems. In the *Drosophila* Set1 knock down, short- and long-term courtship memory were significantly reduced.

While the precise biological pathogenic mechanisms have yet to be clarified, disturbed H3K4 methylation likely underlies this disorder, underscribing the role of H3K4 methyltransferases in memory, developmental disability or neuron function in general.

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P08.55D

Clinical and laboratory management of the largest cohort of Spanish individuals with Phelan McDermid syndrome

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Phelan-McDermid syndrome (PMS; OMIM 606232), results either from the loss of genetic material at the distal region of the long arm of chromosome 22 that including *SHANK3* gene, or by point mutations in *this* gene. *SHANK3* codes for a structural protein, which plays a central role in the formation of the postsynaptic terminals, as well as in the maintenance of synaptic structures. So far, more than 1,200 cases have been already reported; a non-specific phenotype has emerged including global developmental delay (>98%), absent to severely delayed speech (>98%), severe neonatal hypotonia (>98%), normal to accelerated growth (95%), and minor dysmorphic features, among others. We here present the largest cohort of Spanish patients with PMS; 181 (mainly from Spain (151) and Latin-America (30 cases). Patients were characterized by means of deep phenotyping, chromosomal microarrays, and other genetic approaches (including MLPA, FISH and Karyotype). We compare clinical, genetic and follow-up characteristics with previous published series of patients with PMS. In addition, we present clinical-molecular data from this cohort; most of them, with deletions at 22q13.3 band, including *SHANK3* and described 5 new point mutations in this gene associated to PMS. This study set up clinical and laboratory management for the establishment of possible genotype-phenotype correlations. Based on our data and other previously published we propose at first time a complete laboratory management algorithm to verify all the genetic aspects in PMS individuals and related, as well as a correct genetic counselling.

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P08.56A

Functional analysis of a novel c.899+1G>A variant in *SLC9A6* gene

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Introduction: *SLC9A6* (MIM# 300231) is a causative gene for Christianson type syndromic mental retardation, also known as Christianson syndrome, which is inherited in X-linked dominant manner (MRXSCH; MIM# 300243). We provide the results of the functional analysis of a splice site variant c.899+1G>A in the *SLC9A6* gene identified to three affected individuals in one family.

Materials and Methods: *SCL9A6* gene variant NC_000023.11(NM_001042537.1):c.899+1G>A was previously detected by whole exome sequencing in two affected brothers and their mother. To justify the pathogenicity of the variant total blood RNA of one affected male was isolated, template cDNA was synthesized and sequencing of *SLC9A6* gene exons 5-8 was performed by Sanger method.

Results: *SLC9A6* gene donor splice site variant NC_000023.11(NM_001042537.1):c.899+1G>A was examined by computational algorithms and predicted to affect mRNA splicing. Sanger sequencing of cDNA revealed that c.899+1G>A produces skipping of exon 6. *In silico*, this leads to a frameshift in cDNA and results in a premature stop codon NP_001036002.1:p.(Val264AlafsTer3). The truncated protein lacks several transmembrane helices and a C-terminal domain.

Conclusions: Our study demonstrates that *SLC9A6* gene splice site variant NC_000023.11(NM_001042537.1):c.899+1G>A affects mRNA splicing and leads to a truncated protein product, which is the cause of Christianson syndrome in affected family members.

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P08.57B

Identification of compound heterozygous *SNX14* variants in a Portuguese SCAR20 family by a combination of deep phenotyping, exome sequencing and targeted quantitative PCR

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Pathogenic variants in the *SNX14* gene are implicated in Autosomal Recessive Spinocerebellar Ataxia 20 (SCAR20), a rare condition characterized by intellectual disability, lack of speech, ataxia with progressive cerebellar hypoplasia, coarse facies and macrocephaly. We describe a Portuguese family with two siblings presenting similar clinical features caused by compound heterozygous variants in *SNX14*. A heterozygous nonsense variant c.1195C>T p.(Arg399*) was initially identified by exome sequencing. Due to phenotypic similarity with previously published SCAR20 patients, further investigation led to the identification of the second heterozygous variant c.(612+1_613-1)_(1171+1_1172-1) del using targeted quantitative PCR. Reverse transcriptase PCR confirmed the frameshift r.613_1171del p.(Val205Argfs*47). Breakpoint characterization is underway but preliminary results indicate that the underlying mechanism appears to be Alu-induced non-allelic homologous recombination. Our results describe the first non-consanguineous SCAR20 family carrying compound heterozygous pathogenic variants in the *SNX14*. In conclusion, this case emphasizes the role of Sorting nexin-14 in neurodevelopment and cognitive impairment, and highlights the value of detailed clinical evaluation and deep phenotyping to disclose the molecular defect underlying a highly heterogeneous disease such as intellectual disability. Funding: UMIB is supported by National Funds through the FCT - Fundação para a Ciência e a Tecnologia (Portuguese national funding agency for science, research and

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P08.58C

Two *de novo* and one familiar cases of *TLK2*-associated intellectual disability confirm disease variable expressivity

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Introduction: The Tousled-Like Kinase 2 (TLK2) gene has recently been associate with "Mental Retardation Autosomal Dominant 57" (MIM:618050), a neurodevelopmental disorder characterized by a highly variable phenotype, including mild to moderate intellectual disability, behavioural abnormalities, facial dysmorphisms, microcephaly, epilepsy and skeletal anomalies. The role of TLK2 in brain is still unknown. Within the Autism Sequencing Consortium (ASC) project, we identified new patients with variants in the TLK2 gene by whole-exome sequencing (WES).

Methods: DNA samples from trios were sequenced at the Broad Institute on Illumina HiSeq sequencers as previously described (De Rubeis et al., 2014); variant calling was performed using a bioinformatics pipeline adapted for different patterns of inheritance.

Results: We identified one patient with a de novo likely pathogenic TLK2 variant (p.Asp529Gly) and a family with three siblings who inherited a nonsense variant from an affected mother (p.Glu453*). Finally, we report a de novo 39-kb deletion encompassing the TLK2 and MRC2 genes. Clinical phenotypes partially overlapped with known cases: facial dysmorphisms were present in all patients, while intellectual disability and microcephaly were not identified in the patient with the 39-kb deletion, where the skeletal anomalies were predominant.

Conclusion: Our study describes 6 new cases from 3 families with TLK2-associated disease. Our data support the recent report that haploinsufficiency of this gene is involved in heterogeneous forms of intellectual disability.

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P08.59D

Intellectual disability: Identification of novel genes and expansion of genetic and phenotype spectrum by multicentercollaboration

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Intellectual disability (ID), a group of neurodevelopmental disorders with a global prevalence approximating 2-3% with severe ID occurring 0.5-1%, is a frequent reason for referral to pediatric genetics clinics. ID is genetically and phenotypically heterogeneous and can be categorized into syndromic and non-syndromic forms. Although next generation sequencing technologies have uncovered more than a thousand underlying genetic causes, many cases remain undiagnosed. From our cohort of patients with varying degrees of ID, we genetically evaluated 26 families whose probands manifested with syndromic intellectual disability, global developmental delay, and brain malformations. Likely pathogenic variants in known genes were identified in 57% (15/26), novel genetic causes in 20% (5/26) and no pathogenic variants were found in 23% (6/26). For three novel genes of unknown function, matchmaking platforms and multi-center collaboration identified an additional 14 families. One novel gene is TMEM94, in which bi-allelic truncating variants were identified in 10 probands from 6 families; all had syndromic ID, speech delay, facial dysmorphisms and congenital heart defects. We have shown that Tmem94 is essential for neurologic and cardiovascular development in mice. We also demonstrated that, in human cells, TMEM94 is a novel protein localized in the centrosome, and may very well have important roles involved in cell division and metabolism. In conclusion, the identification of novel ID genes is facilitated by matchmaking platforms and collaborative interactions. Efficient analyses using cell and model organisms provide supporting data for diagnosis and understanding of gene function.

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P08.60A

A homozygous loss-of-function variant in the TRAPPC2L gene causes a neurodevelopmental disorder overlapping TRAPPC9-related disorder

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Pathogenic variants in genes encoding proteins belonging to Trafficking Protein Particle (TRAPP) complex have recently been described in several human diseases including intellectual disability.

We report two siblings with a clinical presentation overlapping *TRAPPC9*-related disorder (MIM #613192). Pregnancy and delivery was uneventful. First medical concerns appeared before one year of age due to hypotonia and poor contact. Patients then developed severe intellectual disability with neither independent walking nor speech at current age of 11 and 8 yo. They also had stereotypic hand movements as well as MRI abnormalities, such as thin corpus callosum and cortical atrophy. They had no seizures. Extensive metabolic screening, CGH-array and Intellectual Disability gene panel sequencing were normal. Whole exome sequencing revealed that both affected siblings carried a novel homozygous variant (c.367C>T (NM_016209), p.(Gln123*), hg19:chr16:88926373C>T) in the *TRAPPC2L* gene. The variant was inherited from heterozygous consanguineous parents.

This is the first description of a homozygous loss-of-function variant in the *TRAPPC2L* gene. Only biallelic missense variants in *TRAPPC2L* (*610970) have been previously reported in three patients with global developmental delay, microcephaly, dystonia, tetraplegia, rhabdomyolysis, encephalopathy and epilepsy (Sacher et al. 2018, Milev et al. 2018). TRAPPC2L (Trafficking Protein Particle Complex 2-Like) and TRAPPC9 proteins belong to the human TRAPPII complex, involved in vesicle trafficking in the secretory pathway by mediating contacts between vesicles and target membranes. Our findings suggest that loss-of-function variants in these two genes may cause similar phenotypes. Additional cases are needed to confirm our findings and better delineate the clinical spectrum of this TRAPPopathy.

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P08.61B

Expanding the phenotypic spectrum of *TRIT1* mutatins, a cause of severe autosomal recessive microcephaly

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Introduction: Deciphering genetic basis and identifying new candidate genes in autosomal recessive microcephaly require high throughput sequencing strategies and data sharing. We identified compound heterozygous variants in *TRIT1* gene, encoding a protein involved in i^6A37 tRNA modification. The purpose of our work was to characterize the clinical and molecular spectrum that result from pathogenic *TRIT1* variants. Patients and methods: We used the matchmaking exchange platforms, PhenomeCentral and GeneMatcher to recruit patients with *TRIT1* pathogenic variants. Clinical and molecular data were assessed and compared to the four previously published families.

Results: We identified three new patients with compound heterozygous variants in *TRIT1* gene. All variations were inherited from a healthy parent in heterozygous state. Taking into account published cases, all patients presented moderate to severe ID, seizures and severe microcephaly. Cerebral atrophy or dysplasia of corpus callosum could be noted in half of patients. In most of cases, each compound state associated one missense and one truncating variation. Loss of function variants, considered as pathogenic, presented a mean allele count of 75 in GnomAD database. All but one involved variant were reported many times in heterozygous state in GnomAD [range 2 - 139].

Conclusions: Haplo-insufficiency of TRIT1 causes an emerging clinical syndrome characterized by moderate to severe ID, microcephaly and epileptic encephalopathy. Allele counts for each known pathogenic variation in GnomAD raise the issue of an underdiagnosed cause of microcephaly and/or rapidly lethal associations.

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P08.62C

Functional investigation for Variant of Unknown significance in intellectual disability genes NLGN3, PQBP1 and DYRK1A

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Intellectual Disability (ID) is a neurodevelopmental disorder characterized by significant genetic contribution and heterogeneity. While exome sequencing has revolutionized the identification of novel ID genes and pathogenic variants, a huge challenge that remains is the interpretation of the vast number of Variants of Unknown Significance (VUS). A combination of genetic, clinical and functional arguments is essential for best interpreting these VUS. Development of functional tests to characterize VUS effects are important to make a diagnosis but also to dissect the function of the concerned proteins. We illustrate this question by presenting functional studies performed to reclassify VUS in three ID genes. We first looked at expression and localization of mutant proteins by overexpressing them. For NLGN3, we used the unfolded protein response (UPR) as a read-out as the initial pathogenic variants identified was shown to cause endoplasmic reticulum stress. For DYRK1A, which require an autophosphorylation to be activated, we study the ability of mutant proteins to autophosphorylate themselves. As it was shown that DYRK1A and PQBP1 might regulate gene expression and splicing, we analyzed transcriptomic profiles in patients' cells or by loss-of-function of the genes in human neuronal precursors to sort out molecular signatures. We have been able to reclassify two-thirds of VUS into the pathogenic categories and we are currently developing novel approaches to characterize the effect of the last third. The better comprehension of mechanisms involved in monogenic forms of ID and the development of functional tests for missense variants will be useful to improve diagnosis of patients.

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P08.63D

Pathogenic *WDFY3* variants cause neurodevelopmental disorders and opposing effects on brain size

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Introduction: The underpinnings of mild to moderate neurodevelopmental delay (NDD) remain elusive often leading to late diagnosis and interventions.

Materials and Methods: We present data on exome-, genome-sequencing, and array analysis of thirteen individuals that point to pathogenic, heterozygous, mostly *de novo* variants in *WDFY3* (significant *de novo* enrichment $p = 0.0007$) as a monogenic cause of mild and non-specific NDD.

Results: Nine variants were protein-truncating and four missense. Overlapping symptoms included NDD, intellectual disability (ID), macrocephaly, and psychiatric disorders (ASD/ADHD). One proband presented with an opposing phenotype of microcephaly and the only missense-variant located in PH-domain of WDFY3. Findings of this case are supported by previously published data demonstrating that pathogenic PH-domain variants can lead to microcephaly via canonical Wnt-pathway up-regulation.

We previously reported that Wdfy3 is required for cerebral cortical size regulation in mice, by controlling proper division of neural progenitors. Here, we show that proliferating cortical neural progenitors of human embryonic brains highly express WDFY3, further supporting a role in regulation of prenatal neurogenesis. We present data on Wnt-pathway dysregulation in Wdfy3-haploinsufficient mice, which display macrocephaly and deficits in motor coordination and associative learning, recapitulating the human phenotype. Consequently, we propose that in humans *WDFY3* loss-of-function variants lead to macrocephaly via down-regulation of the Wnt-pathway.

Conclusion: We present *WDFY3* as a novel gene linked to mild to moderate NDD and ID and conclude that variants putatively causing haploinsufficiency lead to macrocephaly, while an opposing pathomechanism due to variants in PH-domain of WDFY3 leads to microcephaly.

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P08.64A

Triple diagnosis of Wiedemann-Steiner, Waardenburg and DLG3-related intellectual disability association found by WES. A case report

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Thank to performance of whole exome sequencing (WES) or whole genome sequencing, it is now possible to identify multiple pathogenic variants in patients with rare disease, where a single disease has until now been suspected. We report the simultaneous discovery of three pathogenic variants in *KMT2A*, *PAX3*, and *DLG3* genes, for a 8-year-old patient. He has a developmental delay, behavioral disorders, associated dysmorphic features, such telecanthus, blue coloring of the irises and cubital hypertrichosis. A trio based exome sequencing found three deleterious variants: *KMT2A:* c.9068delA ;p.Gln3023Argfs*3 de novo, *PAX3:* c.530C>G ;p.Ala177Gly de novo and *DLG3:* c.127delG ;p.Asp43Metfs*22 hemizygous inherited from the mother. *KMT2A* mutations are involved in Wiedemann-Steiner syndrome, and *PAX3* mutations are responsible for Waardenburg syndrome. *DLG3* mutations are described in a non-syndromic X-related intellectual disability. This frameshift variant is located on the specific exon 1 of transcript NM_020730.2 of *DLG3* mostly expressed in brain. Considering the dysmorphic features and intellectual disability presented by this patient, these three variants were therefore imputed as pathogenic and their association responsible for his phenotype. Several multiple molecular diagnosis were already found by WES. Yang *et al*. reported dual diagnosis on 4 patients (6%) [1], Posey *et al*. within 6 patients (7%) [2], and Rossi *& al*. within 4 patients (9.5%) [3]. However none triple diagnosis has been reported in literature. This demonstrates and reminds us of the importance of analyzing exomes in a rigorous and exhaustive manner because it can explain in some cases (<10%) superimposed traits or blended phenotypes.

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P08.65B
Diagnostic Yield and Novel Candidate Genes by Exome Sequencing in 104 Omani Families

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Background: Autosomal recessive inherited neurodevelopmental disorders are highly heterogeneous, and many causative genes are still unknown. Whole exome sequencing (WES) becomes the most efficient test to identify disease-causing variants in monogenic disorders. Aim: To promote the identification of disease genes through WES, hence to estimate the diagnostic yield of WES and to select novel candidate genes for future functional studies.

Materials and Methods: The institutional ethics committee approved this study. 168 patients (104 families) with neurodevelopmental disorders were enrolled and all evaluated by clinical geneticists. For WES; DNA was barcoded and enriched using Agilent SureSelect-V6-60 MB and sequenced using HiSeq4000 or NovaSeq6000. Raw data and Variant Calling Files (VCF) processing and annotation was carried out at the Department of Genetics, SQU.

Results: A database of clinical phenotypes for 104 families was established. In-house pipeline for filtration and prioritization of WES data was standardized and validated. Prioritized variants found were as following 1) disease-causative variants (definite cause) in 21/104 families (20%), 2) possible disease-causing variants (VUSs) with supportive segregations results in 28 families (27%), and 3) variants in novel genes (new genes not implicated in human disease before) in 19/104 families (18%). While in 36 families (35%), we detected no abnormality.

Conclusion: the diagnostic rate achieved in our cohort is 20% when considering pathogenic variants only and up to 47% with VUSs. Nineteen candidate genes that are convincing to be causative of neurodevelopmental syndromes identified. Further functional studies to be completed to confirm causation.

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P08.66C
Variants in the transcriptional corepressor *BCORL1* are associated with an X-linked disorder of intellectual disability, dysmorphic features, and behavioral abnormalities

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Introduction: *BCORL1*, a transcriptional corepressor, is involved in negative gene regulation through associations with several protein complexes including Class II histone deacetylases (HDACs). Acquired somatic mutations in *BCORL1* have been implicated in the pathogenesis of

several malignancies, but germline mutations of *BCORL1* have not been associated with a specific genetic syndrome. However, a hemizygous variant, c.2459A>G [p.(Asn820-Ser)] in *BCORL1* was reported in 2 brothers with severe intellectual disability, coarse facial features, and hypotonia previously.

Materials and Methods: Five individuals from 3 pedigrees with phenotypes including intellectual disability, behavioral difficulties, and dysmorphic features were investigated by whole exome sequencing. Our case series was built via collaborations assisted by GeneMatcher.

Results: Three missense variants c.2345 T>A, c.1487 C>T and c.95C>T were observed in hemizygous state in *BCORL1*. Two of the three *BCORL1* variants, c.95C>T [p.(Pro32Leu)] and c.1487C>T [p.(Ser496Phe)] are not reported in gnomAD and the third variant, c.2345T>A [p.(Val782Glu)] is reported in one individual in hemizygous state. The amino acids, proline at 32nd and serine at 496th position are highly conserved with a GERP score of 5.42 and 5.69 respectively. The amino acid, valine at 782th is moderately conserved with a GERP score of 2.7.

Conclusions: Hemizygous pathogenic variants in *BCORL1* underlie a new X-linked epigenetic syndrome of variable degrees of intellectual disability, seizures, behavioral abnormalities, and dysmorphisms including tall forehead, hypertelorism, downslanting palpebral fissures, and long fingers. This newly described syndrome should be considered in males with the above described features, especially in the setting of an X-linked familial inheritance pattern.

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P09.001D
The rare 13q33-q34 microdeletions - eight new patients and review of the literature

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Introduction: Deletions of 13q33-q34 cytoband are rare chromosomal aberrations, and literature evidence describing the clinical characteristics is scarce. Our objective was to shed light on the phenotype and inheritance pattern of this unique microdeletion.

Methods: Cases with deletions involving the 13q33.1-q34 cytoband were retrieved using local databases of two largest Israeli centers performing CMA analysis. In addition, literature search in PubMed database and DECIPHER database was performed.

Results: Local database search yielded eight new patients with 13q33.3-q34 microdeletions, three of which had additional copy number variants. Combined with 15 cases detected by literature search, and additional 23 cases reported in DECIPHER database, overall 43 patients with isolated 13q33.3-q34 microdeletions are described. Developmental delay and/or intellectual disability were noted in the vast majority of affected individuals (93.2%), and in all cases extending beyond the 13q34 cytoband (n=27). Of the 20 deletions involving the 13q34 cytoband only, in three cases developmental delay and/or intellectual disability was not reported. Interestingly, in two of these cases (66.7%) the deletions did not involve the terminal *CHAMP1* gene, as opposed to 3/17 (17.6%) of patients with 13q34 deletions and neurocognitive disability. Facial dysmorphism and microcephaly were reported in about half of the cases, while convulsions and heart anomalies were noted in one fifth of the patients. None of the 13q33-q34 deletions were inherited from a reported healthy parent.

Discussion: 13q33-q34 microdeletions are associated with high risk for neurodevelopmental disability. The rarity of this chromosomal aberration necessitates continuous reporting and collection of available evidence.

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P09.002A
New name? CD59-related immune-mediated polyneuropathy with/without hemolytic anemia?

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Introduction: Hemolytic anemia, CD59-mediated, with or without immune-mediated polyneuropathy is a very rare autosomal recessive disease, adding to chronic hemolytic anemia, infantile-onset polyneuropathy attacks often triggered by an acute infection. Here, a previously defined mutation in a few cases in the literature will be discussed.

Methods: The girl's DNA was analyzed by tru sight one sequencing. Target sequence analysis was performed to her patient brother and healthy parents.

Results: A 14-year-old girl applied to us with neurologic attacks as muscle weakness especially in the right side,

decreased vision, painful eye movements after acute infection started at the age of 4. Her 11-year-old brother had neurologic attacks that began at the age of 15 months, with lower extremity muscle weakness, tenderness sleep, walking-standing up difficulty. During the attacks, MRI findings were consistent with ADEM/transverse myelitis; hematuria and mild anemia with negative direct-indirect coombs tests was found only once. They benefited from IVIG and steroid treatments. In their physical examination; ptosis, atrophy, decreased deep tendon reflexes, superficial sensory loss in the right side of the girl's body and face; decreased muscle strength, superficial sensory loss, absence of deep tendon reflexes in the boy's lower extremities were detected. Homozygous pathogenic c.146delA (p. Asp49Valfs * 3) mutation was detected in the girl's CD59 gene. The same mutation was found as homozygous in her brother, heterozygous in their parents who had a consanguineous marriage.

Conclusions: This family presents new data to the literature because of the beginning age, shape of neurological attacks and absence of chronic hemolysis.

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P09.003B
A first report of homozygous missense mutation in the ADCY5 gene related to an autosomal dominant Dyskynesia syndrome

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Background: ADCY5-Related Dyskinesia is a diverse group of movement disorders, characterized by dystonia, myoclonus or chorea, with variable disability. The disorder is inherited in an autosomal dominant manner; however, recently two siblings with generalized dystonia and myoclonus have been reported, associated with compound heterozygous mutations in ADCY5 gene.

Patient: We describe girl, born to consanguineous parents (first cousins) following an uneventful delivery at term. At the age of 1 year and 9 months she presented a severe global developmental delay, and was unable to speak, sit or stand. Her neurologic examination showed markedly increased tonus and episodic dystonic postures in trunk, extremities and face, exacerbated by voluntary movement. In addition, spontaneous events of ocular convergence spasm and orofacial dystonia were observed. However, during sleep a significant decrease in the tonus was noted, with fill range of joint movements. She had recurrent episodes of hyperthermia and hyperhidrosis. Magnetic resonance imaging was suggestive of delayed myelination, and metabolic workup was non-directive.

Results: Whole exome sequencing revealed a novel homozygous c. 1406G>A (p. Ser469Asn) mutation in the ADCY5 gene (OMIM 600293). The variant was not found in GnomAD or ExAC browsers, was classified as damaging/disease causing by in-silico prediction programs, and the residue Ser469 was shown to be highly conserved throughout evolution. Both parents, reported as healthy, were found to be heterozygous carriers.

Conclusion: To our best knowledge, we present a second family with biallelic mutations in ADCY5 gene, associated with a severe neurologic presentation of ADCY5-Related Dyskinesia spectrum.

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P09.005D
Polygenic risk score improves prediction of alcohol-related morbidities

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Alcohol drinking is a major contributor to global health burden. Affected by genetic factors, genome-wide association studies (GWAS) have identified multiple loci associated with alcohol consumption. To quantify the prognostic information of this polygenic burden, we derived a polygenic risk score (PRS) for alcohol consumption and tested its association with alcohol use disorder, alcohol-related morbidities and mortality.

We created 21 major alcohol-induced health events using nationwide hospital discharge, cause-of-death and prescription drug purchase registries. Using the largest GWAS summary statistics on alcohol consumption (GSCAN, n=527,282 non-Finns), we built a genome-wide PRS and tested its prediction on alcohol-related events in prospective cohorts (total n=39,243).

Per 1SD increase, the PRS was associated with a 11.2g (0.93 drinks) increase in weekly alcohol intake (95% CI=9.85-12.58g, $p=2.3*10^{-58}$) and a 27% increase in alcohol-induced events (cases=874, HR=1.27,1.19-1.36, $p=1.7*10^{-12}$) while the difference between the risk of the lowest and highest PRS-quintiles was 102% (HR=2.02, 1.62-2.52, $p=5.4x10^{-10}$). Controlling for self-reported alcohol-consumption-estimate, socioeconomical status, smoking, and GGT, this increase was 17%/SD (1.17, 1.09-1.25, $p=8.9*10^{-6}$). For DSM-IV AUD, a similar increase was observed (cases=713, OR=1.20, 1.11-1.31, $p=2.29*10^{-5}$). Adding the PRS over age and sex increased the C-index 2% (p=0.017) and self-reported consumption and socioeconomic factors increased the C-index further by 13% ($p=3.1*10^{-11}$) with the C-index for a model including all being 84.9%.

In conclusion, increased polygenic risk for alcohol consumption was associated with alcohol-related morbidities also when controlling for self-reported alcohol consumption and other covariates thus showing potential in utilizing genetic information for prediction of alcohol-related harms.

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P09.006A

Telomere length and mitochondrial DNA copy number changes as a biomarker in ALS

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Introduction: Telomere length was previously shown to be decreased in several neurodegenerative disorders while mitochondrial DNA copy number changes were reported to show conflicting results. mtDNA copy numbers and telomere length are reported to be correlated. Bigger sample sizes, simultaneous measurements and detailed data on disease severity is lacking.

Materials and Methods: ALS patients (n=99) were staged based on the King's clinical staging system (Stage I-IV.). mtDNA copy numbers and telomere lengths were measured with real-time PCR technique.

Results: Stage I ALS patients have an average telomere length of 200.6 (kB) (SD: 69.5), mtDNA copy number of 300.3 (SD: 90.3), while stage II telomere length is 164.9 (SD: 77.9) and mtDNA copy number is 280.7 (SD: 85.9) and stage III telomer lengths is 114.1 (SD: 40.5) while mtDNA copy number is 256.5 (SD: 93.9). We did not have any stage IV patients.

Conclusions: Both telomere length and mitochondrial copy number decreased with disease severity. No significant difference between sporadic and familial forms have been found. Changes in the telomere length are significant ($p<0.05$) and telomere length appears to be a better biomarker in late stages. Grant: NTP-NFTÖ-17-B-0595

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P09.007B

Next Generation Exome Sequencing in a Large Sample of Alzheimer's Patients

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Introduction: Both early- and late-onset types of Alzheimer's disease (EOAD and LOAD) have a substantial genetic component. Despite the high genetic heritability, a large proportion of the risk has not been explained thus far. A considerable proportion of the missing heritability is likely to be accounted for by rare, low frequency and functional variants. Here we apply whole exome sequencing (WES) to identify rare genetic variants that influence disease risk.

Methods: We are currently sequencing a large cohort of both EOAD (2000 individuals) and LOAD patients (3000) and healthy elderly controls (1100). WES is performed using Illumina HiSeq 4000 at a median coverage 40x. The GATK pipeline is utilised for variant discovery. The quality control and statistical analyses are performed within the Hail framework. We will perform single-variant association analyses as well as gene burden analyses. In addition, we will check if there are rare variants in the genes previously associated with rare forms of dementia.

Results: We will present our first large scale genomic analysis results based on the WES data for the cohort at the ESHG2019.

Conclusions: To identify novel genetic causes of AD, we are sequencing a large cohort of both EOAD and LOAD

patients. Our analysis will utilise this well powered sample to identify novel low frequency loci in addition to functional variation that have been potentially missed by GWAS. Identification of these loci will further elucidate the genetic architecture of EOAD and LOAD and will implicate functional variants for molecular investigation and potential drug targets.

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Effect of *APOE* on cognitive function and dementia in a longitudinal cohort of 13,131 healthy elderly individuals

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Introduction: *Apolipoprotein E* (*APOE*) is associated with cognition, longevity, cardiovascular disease, Alzheimer's disease (AD) and all-cause mortality. Homozygosity for the Ɛ4 allele increases AD risk, with homozygotes estimated having up to 60-70% lifetime risk (penetrance), with average age of onset ~70 years. Some Ɛ4 homozygotes live >75 without AD, suggesting protective factors, yet are rarely ascertained. We sought to test *APOE* haplotypes against cognitive function/dementia in a longitudinal study and identify Ɛ4 homozygotes aged >75 years without AD.

Methods: We sequenced 13,131 healthy elderly participants in the ASPREE study. At baseline, participants had no history of cardiovascular disease or dementia, and passed a general cognition screen (Modified Mini-Mental State Examination ≥78), average age 74 years. We administered different cognitive tests (general cognition, verbal learning/ memory, verbal fluency, processing speed) over five-years. We collected clinical outcome data on a range of endpoints, and associated each *APOE* haplotype with specific cognitive functions and dementia.

Results: *APOE* frequencies aligned with other cohorts (Ɛ3/Ɛ3=62.3%, Ɛ3/Ɛ4=21.0%, Ɛ2/Ɛ3=12.5%, Ɛ2/Ɛ2=0.6%, Ɛ4/Ɛ4=1.5%). Yet we observed notable differences in cognitive function (verbal learning/ memory), after controlling for demographic and clinical risk factors. We quantified the effect of each *APOE* haplotype on dementia risk and age-of-onset. We identified 200 Ɛ4 homozygous, of whom after median 4.7 years follow-up, only 14 reached the dementia endpoint (7% penetrance). The average age of the remaining 186 individuals was 78 years, suggesting an enrichment of unaffected individuals in this study.

Conclusion: This uniquely ascertained population provides a platform for discovery of protective genetic factors

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Evaluation of a novel variant in *CR1* in patients with Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative dementia in the elderly. According to the World Alzheimer's Report 2018, 50 million people are living with dementia and its total estimated cost is US$1 trillion worldwide. This poses a significant burden to the aging population.

Previous studies have identified genes that increase the risk of the late onset form of the disease (LOAD). Among them *CR1*, a gene encoding a receptor in the complement system. By sequencing of exomes of Spanish family members affected by AD, we identified a novel variant of *CR1* gene encoding for a truncated protein caused by a single nucleotide variant (SNV). Allele specific PCR revealed the presence of this SNV in 3 members of the family, affected by AD but not the unaffected member nor 192 controls examined nor any public database. These results together with the implication of the gene in the disease, indicates possible pathogenic significance. Plasmid constructs of two most common isoforms and the novel variant of *CR1* fused with Flag epitope were used in an assay to determine whether the variant mRNA is degraded by nonsense-mediated mRNA decay (NMD). Also whether, if translated, the stability of the protein is affected. Preliminary results suggest the novel variant in *CR1* is degraded by NMD as the inhibition of this pathway increases the levels of *CR1* mRNA and protein.

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P09
Neurogenetic and psychiatric disorders

P09.010A
Genetic and clinicopathological contribution of rare *ABCA7* mutations in Belgian early-onset Alzheimer's disease patients

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Introduction: Genetic studies in early-onset Alzheimer's disease (EOAD) patients suggested an important role for premature termination codon (PTC) mutations in *ABCA7*. *ABCA7* was initially associated with late-onset Alzheimer's disease (AD) in genome-wide association studies. PTC mutations are predicted to lead to loss of functional protein though active transcript rescue was observed.

Materials and Methods: Targeted resequencing of the *ABCA7* coding region or whole exome sequencing were used to determine the frequency of *ABCA7* PTC mutations in 757 EOAD patients (mean onset age 61.2±7.0 years) and 757 control individuals. Clinicopathological characteristics of mutation carriers were retrospectively reviewed.

Results: We identified 13 different *ABCA7* PTC mutations in 34 carriers (34/757, 4.49%) and 15 PTC mutations in controls, resulting in an OR of 2.33 (95% CI [1.26-4.31], $p = 0.006$). Carriers had a mean onset age of 61.6±5.8 (48-70) years. Clinical presentation was predominantly amnestic. A positive first-degree familial history was present in 88.8% (16/18). Neuropathological examination (n=5) showed hallmark AD lesions, in 80% (4/5) combined with pronounced cerebral amyloid angiopathy (CAA). Missense mutations were enriched in patients versus controls (OR of 2.20, 95% CI [1.06-2.85], $p = 0.028$).

Conclusion: PTC mutations in *ABCA7* are relatively frequent in Belgian EOAD patients, particularly in familial EOAD. Clinical and neuropathological data exhibited a classical AD phenotype in combination with CAA and highly variable onset ages. Additional information of mutation frequency and spectrum as well as biological impact is essential before implementation into clinical practice, including genetic testing and risk prediction.

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Anorexia nervosa genome-wide association study identifies eight loci and implicates psychiatric and metabolic origins

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Introduction: Anorexia nervosa (AN) is an eating disorder characterised by severe weight loss either through caloric restriction or heightened energy expenditure or a combination of both. It has one of the highest mortality rates of all psychiatric disorders and psychological and pharmacological treatments show only limited effect.

Materials and Methods: As an international collaboration between the Anorexia Nervosa Genetics Initiative and the Psychiatric Genomics Consortium, we performed the worldwide largest genome-wide association study (GWAS) amassing 16,992 AN cases and 55,525 controls. Secondary analyses included chromosome conformation capture (i.e., Hi-C), linkage disequilibrium score regression, generalised summary data-based Mendelian randomisation (GSMR), gene, and gene-set analysis using MAGMA.

Results: The GWAS identified 8 independent loci and estimated its common genetic variant heritability to be 11-17%, indicating a polygenic trait. Enrichment analyses implicate central nervous tissues and cell types in AN. Additionally, AN showed positive genetic correlations with obsessive-compulsive disorder (OCD, $r_g = .45$), major depressive disorder ($r_g = .28$), and anxiety ($r_g = .25$), mirroring its clinical comorbidity profile. Surprisingly, bidirectional GSMR a showed significant bidirectional relationship between AN and BMI ($\beta;;;;;_{AN\rightarrow BMI} = -0.20$; $OR_{BMI\rightarrow AN} = 0.96$), indicating that genomic variants that predispose to lower body mass may also increase liability for AN. Complementing these findings, we also reported genomic overlap between AN and metabolic traits, such as fasting insulin concentrations ($r_g = -.24$) as well as high-density lipoprotein concentrations ($r_g = .21$).

Conclusions: Through these findings, we are encouraging a reconceptualization of AN as both a psychiatric and metabolic disorder.

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The usefulness of array comparative genomic hybridization in detection of copy number variants in patients with epilepsy

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Introduction: Epilepsy is one of the most common diseases of the central nervous system affecting about 1% of the human population. The causes of epilepsy, because of the clinical and etiological heterogeneity, often remain unknown. However, it is well known that genetic factors are responsible for epilepsy in 40-60% cases. Recent studies have shown that in addition to the point mutations, the copy number variants (CNVs) are also important factors in this disorder.

Materials and Methods: We used array CGH method in a group of 54 patients with the clinical diagnosis of epilepsy and neurodevelopmental abnormalities with or without dysmorphic features. Array CGH analysis was performed using genome-wide microarray with average resolution of 30 kb (180K, Oxford Gene Technology) with specifically designed coverage for over 212 selected genes, known or candidate to play an important role in the pathogenesis of epilepsy.

Results: Chromosomal microarray analysis revealed 20 CNVs in 17 patients (31%). All of the identified CNVs were submicroscopic in size, ranging from 1.7 kb to 3.84 Mb, and therefore could not have been detected by standard karyotype analysis. We identified 6 pathogenic or potentially pathogenic CNVs (11%) and 14 CNVs with unknown clinical significance (26%). 10 CNVs (50%) could not have been detected by our clinical microarray (60K, OGT).

Conclusions: The results of our studies further support the role of submicroscopic CNVs in the etiopathogenesis of epilepsy and demonstrate the usefulness of array CGH in the genetic diagnosis of this neurodevelopmental disorder.

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P09.014A
Elucidating the genetic background of childhood-onset ataxias

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Introduction: Despite available genetic testing, a large proportion of all documented ataxia cases remain genetically uncharacterized. Childhood-onset ataxias are clinically and genetically heterogeneous, which makes finding the molecular diagnosis challenging. The aim of this study was to characterize the genetic background of childhood-onset ataxias in Finland using WES technology.

Methods: Our cohort includes all pediatric patients with ataxia as the primary symptom of disease evaluated in Helsinki University Central Hospital during the years 1999-2016. Patients with acute, infection related ataxias, ataxias that follow brain insult and patients with mild ataxia as a minor part of a disorder were excluded. 42 families lacked a genetic diagnosis and were investigated using WES.

Results: A pathogenic or likely pathogenic mutation was found for 17 families (40 %). Known or novel autosomal recessive variants were found in known ataxia genes HIBCH, STUB1, ADCK3, B9D1, CLN5, PTRH2, TPP1 as well as in the novel ataxia gene SQSTM1, encoding autophagy receptor p62, we reported recently. De novo or dominant variants explained approximately a third of genetic causes in our cohort, with variants identified in EBF3, ITPR1, NKX2-1 and ATP1A3. A de novo variant was identified in MED23, which has not previously been linked to ataxia.

Conclusions: WES is an effective way to diagnose patients with known and novel causes of childhood-onset ataxia, and enables early diagnosis, which is critical for patients with treatable forms of genetic ataxia and for genetic counselling. Furthermore, our findings expand the genetic spectrum of childhood-onset ataxias and highlight novel cellular mechanisms.

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Ataxia telangiectasia-like disorder in a family deficient for MRE11A, caused by a *MRE11* variant

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Objective: We report three siblings with the characteristic features of ataxia-telangiectasia-like disorder associated with a homozygous *MRE11* synonymous variant causing nonsense-mediated mRNA decay and MRE11A deficiency.

Methods: Clinical assessments, next-generation sequencing, transcript and immunohistochemistry analyses were performed

Results: The patients presented with poor balance, developmental delay during the first year of age and suffered from intellectual disability from early childhood. They showed oculomotor apraxia, slurred and explosive speech, limb and gait ataxia, exaggerated deep tendon reflex, dystonic posture and mirror movement in their hands. They developed mild cognitive abilities. Brain magnetic resonance imaging in the index case revealed cerebellar atrophy. Next-generation sequencing revealed a homozygous synonymous variant in *MRE11* (c.657C>T, p.Asn219=) that we show affects splicing. A complete absence of *MRE11* transcripts in the index case suggested nonsense-mediated mRNA decay and immunohistochemistry confirmed the absence of a stable protein.

Conclusions: Despite the critical role of MRE11A in double-strand break repair and its contribution to the Mre11/Rad50/Nbs1 complex, the absence of MRE11A is compatible with life.

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Novel intronic and synonymous variants: validation of bioinformatic splicing effect prediction increases the molecular diagnosis rate

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The correct interpretation of biological consequences of variants of unknown significance (VUS) on splicing poses a major challenge for clinical geneticists in molecular diagnosis. *In silico* tools can be used to predict the functional impact of a given VUS; however, software limitations have not been properly evaluated and final confirmation requires further molecular studies. Therefore, our aim was to evaluate the functional impact of two novel VUSs (intronic c.1580-18C>G in *ATP8A2* and synonymous c.6819G>T in *NIPBL*) on splicing, by a minigene assay, and compare the observed effects with the predictions obtained through bioinformatics tools. Minigene constructs were generated through PCR amplification, from patient´s DNA, of genomic sequences surrounding the VUS of interest, which were cloned into the pCMVdi vector. Altered splicing was evaluated by PCR and Sanger sequencing of cDNA obtained from HEK293T cells expressing the minigene constructs. This strategy showed that both variants produced aberrant transcripts. The c.1580-18C>G homozygous variant in *ATP8A2* leads to the retention of 17bp of intron 17, by the use of an alternative acceptor splice site, resulting in a premature termination codon in the predicted protein sequence. The *NIPBL* variant, c.6819G>T, leads to a deletion of 137bp in exon 40, through activation of an exonic cryptic donor splice site, also resulting in a premature termination codon. This allowed us to clearly classify these VUS as disease-causing, confirming the bioinformatics prediction of their functional impact. Furthermore, this work denotes the importance of considering intronic and synonymous variants as a way of increasing the molecular diagnosis rate.

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A novel CDKL5 mutation in an atypical Rett syndrome patient and development an efficient method for detecting CDKL5 activity

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Rett syndrome (RTT) is a severe X-linked dominant inheritance disorder with a wide spectrum of clinical manifestations. Mutations in Methyl CpG binding protein 2 (MECP2), Cyclin dependent kinase-like 5 (CDKL5) and Forkhead box G1 (FOXG1) have been associated with classic and/or variant RTT. This study was conducted to identify the responsible gene(s) in atypical RTT patient, and to examine the effect of the mutation on protein function. DNA sequence analysis showed a novel heterozygous mutation in CDKL5 identified as c.530A>G which resulted in an amino acid substitution at position 177, from tyrosine to cysteine. We demonstrated *in vitro* kinase assay using radioisotope (RI) of mutant protein showed impairment of its activity. The results suggested the mutant CDKL5 was responsible for the disease.

Next, pathogenic point mutations including Y177C are mostly observed within the catalytic domain of CDKL5, therefore loss of catalytic activity may be related to disease onset. However, this hypothesis has rarely been demonstrated. We develop an efficient method for detecting CDKL5 activity. Appropriately, CDKL5 underwent autophosphorylation following expression in *Escherichia coli*, with autophosphorylated CDKL5 detected as a band shift by phos-tag SDS-PAGE, without enzyme purification and RI. We tried to examine the effect of 15 pathogenic or likely pathogenic or uncertain significant mutations on their activity, we found all variants showed dramatically reduced catalytic function. Thus, the protocol is useful for examining the relationship between disease-causing mutations and their activity.

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Autism monozygotic twins with APBA2 gene duplication vary for tick development

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Introduction: Autism, or autism spectrum disorder (ASD) is neurological and developmental disorder that begins early in childhood and lasts throughout a person's life, characterized by challenges with social skills, repetitive behaviors, speech and non-verbal communication. Large genome rearrangements are being identified in ASD as an underlying cause for development of this syndrome. These rearrangements are mostly deletions, but duplications can be found. We present case of twin boys with APBA2 gene duplication.

Materials and Methods: Twin boys, age 9 were referred to genetic counseling and testing with diagnosed ASD (DSM-IV). DNA was isolated from buccal swabs of the boys, and 3 ml of whole blood from parents. Parents provided completed questionnaires for tick disorders in children as well as Coordination Disorder Questionnaire (DCDQ). Identification of mutations was made with MLPA (multiplex ligase-dependent probe amplification) technique, with MLPA kits P343 and P339 (Mrc-Holland).

Results: All samples were typed successfully. We identified de novo APBA2 gene duplication in both twins. This gene, located on 15q13.1 locus is already associated with autism features. DCDQ scores are similar in twins with indication of DCD. Assessment of the Questionnaires for tick disorders in children revealed that one sibling is completely absent for ticks while other has high score for section related to vocal and involuntary ticks.

Conclusions: This finding require further investigation and follow up on spatial and temporal differences in autism associated traits in APBA2 gene duplication associated autism.

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P09.019B

MLPA analysis as a diagnostic test in patients with autism spectrum disorders

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Introduction: Autism spectrum disorders (ASDs) are a heterogeneous group of conditions characterized by impaired reciprocal social interaction, lack of communication, isolated interests and repetitive or stereotyped behaviors. Most cases are idiopathic, although there is increasing evidence that ASDs have an important genetic component with aetiological heterogeneity. The aim of our study was to evaluate the role of multiplex ligation-

dependent probe amplification (MLPA) as a screening test in patients with autism spectrum disorders.

Materials and Methods: For this study we used MLPA P245 Microdeletion Syndromes for screening of the most common microdeletion syndromes and MLPA P036 Subtelomeres Mix 1 for screening of subtelomeric deletions/ duplications in 198 patients with autism spectrum disorders. To confirm alternations discovered with MLPA P036 Subtelomeres Mix 1 we used MLPA P070 Subtelomeres Mix 2B.

Results: We identified 12 autistic patients with submicroscopic aberrations. There were 2 patients with subtelomeric deletion at the 14q and 2q regions respectively. Two patients had the same deletion at the 1p36.33 region. One patient had a submicroscopic deletion at the 6q region and duplication at the 13q region simultaneously. Two patients had the submicroscopic deletions at the 4q region. Three duplications were detected at 3p, 9p, and 17p11.2 regions. Two patients were with deletion at the 8p and 22q11.21 regions respectively.

Conclusions: The present study shows that the incidence of a submicroscopic aberrations detected by MLPA in autistic patients is approximately 6 %. MLPA is a rapid and cost effective method for detection of genomic imbalances in patients with autism spectrum disorders.

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P09.020C
Rare variants targeting genes that encode for cytochrome P450 enzymes in Autism Spectrum Disorder

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Autism Spectrum Disorder (ASD) heritability estimates of 50-80% support the hypothesis that gene-environment interactions play a role in this pathology. ASD risk has been associated with early exposure to various xenobiotics that are cleared in the liver by Cytochrome P450 enzymes. We therefore explored the hypothesis that variants in *CYP450* genes, which define the rapid or slow metabolizer status of their carriers, may render individuals exposed to certain toxicants more susceptible to brain disruption during early development. To identify rare variants in 57 *CYP450* genes we inspected exome sequence data, from publicly available ASD datasets, for loss-of-function (LoF) and missense Single Nucleotide Variants (SNVs) predicted to be detrimental by *in silico* tools (MAF<5%), as well as rare (<1%) Copy Number Variants (CNVs). In 2674 ASD subjects, we found 516 LoF and missense SNVs, targeting 54 *CYP450* genes, most frequently in *CYP2A13*, *CYP1A1*, *CYP2D6* and *CYP4B1*. Additionally, we identified 135 CNVs targeting 24 *CYP450* genes in 3030 ASD individuals. *CYP2D6* was exclusively targeted by CNVs in 13 out of 3030 ASD-subjects, while *CYP4X1* was significantly more frequently targeted by CNVs in ASD cases (22/3030; 0.72%) than controls (2/9649; 0.02%). *CYP2D6* metabolizes most psychoactive drugs, while *CYP1A1* acts on polycyclic aromatic hydrocarbons (PAHs) and *CYP4X1* is involved in the metabolism of endocrine-disrupting chemicals (e.g. phthalates and bisphenol A). Early exposure to neurotoxic phthalates, bisphenol A and PAHs has been previously associated with ASD risk, and here we provide evidence for an effect of gene-environmental exposure interaction mediated by *CYP450* gene variants.

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P09.021D
Autism Spectrum Disorder: gene variants involved in the nonsense-mediated mRNA decay pathway

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Genetic factors account for 50-80% of the familial risk of Autism Spectrum Disorder (ASD), but most of the genetic determinants are still unknown and a role for other regulatory mechanisms is likely. The nonsense-mediated decay (NMD) pathway is essential to control mRNA quality and has an important role in the regulation of the transcriptome. Mutations in genes involved in the NMD pathway, such as the *UPF3B* gene, a core component of this pathway, were previously linked to ASD. In this study we explored the potential role of other NMD factors in ASD.

We generated a list of 153 genes involved in the NMD pathway using AmiGO, Reactome and a systematic literature review. To identify potentially pathogenic variants in the NMD genes, we analyzed whole exome sequencing data (WES) data from 1338 ASD subjects. We also searched for Copy Number Variants (CNVs) targeting NMD genes in ASD patients (n=3570) and checked their frequency in controls (n=9649).

We identified 43 high impact variants in 28 NMD genes, including the *UPF3B* and *ACE*, two genes previously implicated in ASD. Importantly, 11 were novel candidate genes that carry loss-of-function and missense (deleterious and damaging) variants with a frequency of 1 to 5% in this ASD dataset. Additionally, 5 NMD genes were found to be targeted by CNVs in 12 ASD subjects but none of the controls.

The discovery of 33 NMD genes that are intriguing candidates for ASD in large patient genomic datasets supports the involvement of the NMD pathway in ASD pathophysiology.

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P09.022A
Application of oligonucleotide array CGH in 280 patients with autism spectrum disorder

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Autism Spectrum Disorders (ASD) is a heterogenous group of neurodevelopmental disorders characterized by problems in social interaction and communication as well as the presence of repetitive and stereotyped behaviour. It is estimated that the prevalence of ASD is 1-2% in general population with the average male to female ratio 4-5:1. Research studies have shown that clinically relevant CNVs (copy number variants) invisible in karyotype analysis are detected in 7-14% of patients with idiopathic ASD.

We elected to use oligonucleotide microarrays (OGT) with average resolution of 30 kpz to evaluate its efficacy for identification and characterization of CNVs in a cohort of 275 patients with ASDs. The analyses of the patients' genomes were performed using exon-focused, high-resolution (180k) array design covering relevant 227 genes for autism research.

Chromosomal microarray analysis revealed 71 non-polymorphic CNVs in 63 out of 280 (22.5%) patients with ASD. Pathogenic or likely pathogenic CNVs were detected in 24 (8.6%) patients, whereas CNVs with unknown clinical significance were identified in 41 (18.2 %) of cases. All of the identified CNVs were submicroscopic in size (between 15 kb and 3.1 Mb) and therefore could not have been detected by standard karyotype analysis. Due to high resolution of the selected microarray, it was possible to identify 24 CNVs that could not have been detected using the clinical microarrays (OGT, 60k). Our study further confirmed the potential of aCGH in elucidating the etiology of ASDs, demonstrated by the identification of two novel genes: LRRTM4 and DOCK1 as candidate for ASDs.

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P09.024C
Genetic determinants for social skill training outcomes in autism spectrum disorder

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Introduction: Social skill group training (SSGT) is one of the most common interventions for children and adolescents with autism spectrum disorder (ASD). Individual responses to SSGT vary and limited clinical predictors exist for the treatment.

Material and Methods: Therefore, we performed a detailed genetic characterization using genotyping, CNV calling and exome sequencing of autistic individuals from one of the largest randomized clinical trial for SSGT and analyzed the association between genetic factors and SSGT treatment outcome. Identified rare copy number variations (CNVs) were prioritized and polygenic risk score (PRS) was calculated from ASD, education attainment (EA) and attention deficit hyperactivity disorder (ADHD) based on different p-value thresholds (Pt < 0.01, 0.05, 0.1, 0.5, 1).

Results: Individuals who carried large CNVs (> 500kb) showed significant worse outcome at 12 weeks post-treatment ($\beta = 15.4$, $p = 0.017$) and 3-months follow-up ($\beta = 14.2$, $p = 0.028$). In addition, inferior outcomes were implicated for individuals with higher PRS for ASD (Pt = 0.5: $\beta = 6.5$, $p = 0.018$) and ADHD (Pt 1.0: $\beta = 6.7$, $p = 0.015$) at follow-up treatment. Currently, we are analyzing exome sequencing data from the same 205 individuals and then combining different rare and common genetic variant data carriers together.

Conclusion: Autistic individuals with higher genetic burden for the disorder, including large rare CNVs and higher load of PRS, have different benefits of SSGT compared with individuals with lower genetic risk. Our results can aid in personalized intervention modifications for ASD in the future.

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P09.025B
Evidence for altered calcium signaling and altered mitochondrial function in an autism case study

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Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by deficits in social interaction, communication, and stereotypic behaviors. While its etiology is unknown, the large assemblage of risk variants impacting calcium ion channels and signaling proteins suggest that a functional disruption of this signaling hub may be involved in ASD pathogenesis. In this study, we evaluate how such risk variants exert their deleterious effects in a unique ASD case.

We identified this subject in a previous study where we observed significant changes in the single-channel inositol triphosphate (IP3) receptor kinetics of ASD fibroblasts via "optical patch-clamp" and found that this feature could be visualized with a high-throughput Fluorometric Imaging Plate Reader (FLIPR) screening assay as a decrease in calcium release from the ER. This release was below the lower limit of controls in >75% of ASD subjects, but uniquely high in an 18-year old autistic female at a level far exceeding the upper limit of controls, nearing levels obtained with ionomycin, an ionophore.

To assess the molecular basis of this finding, we completed a transcription analysis to compare expression levels of calcium signaling-related genes in fibroblast-derived RNA from this subject to those of two controls and two typical autism cases. The subject's transcriptome showed increased expression (>5sd) of genes including the ATP2A3 calcium pump and purinergic receptors, and extremely low expression (>24sd) of VDAC2, a mitochondrial calcium uptake channel. These findings corroborate suggestions of mitochondrial dysfunction in her clinical biochemical assays and are extended with Seahorse XFp assays.

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P09.026A
Establishing genotype-phenotype associations for ASD

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Genotypic and phenotypic heterogeneity of Autism Spectrum Disorder (ASD) has hindered the establishment of genotype-phenotype associations. Herein, we presented a novel approach that integrates semantic similarity and unsupervised machine learning methods to dissect the ASD genotypic heterogeneity and to identify phenotypic manifestations of ASD genetic variants. This approach was applied to copy number variants (N=6650), disrupting 3998 genes from 1119 ASD patients. Functional similarities among genes were computed using Resnik semantic similarity measure. Semantic similarity score, ranging from 0 to 1 represents the functional similarity between two genes, where 1 represents identical genes while score 0 reflects functionally dissimilar genes. Agglomerative hierarchical clustering of the computed gene similarity matrix identified four different clusters of functionally related genes. Silhouette analysis indicated that clusters were compact and consistent (average Silhouette value=0.31). The genes (N=519) of cluster 1 was more relevant to ASD as they were enriched for Cell adhesion molecules (CAMs) (adjusted p-value=0.00001) and Axon guidance (adjusted

p-value=0.02) pathways, which are known to be strongly associated with ASD. Cluster 1 genes were also most significantly enriched for Schizophrenia Human Phenotype Ontology (HPO) term, which is a co-occurring condition with ASD. The other three clusters were not enriched for ASD related HPO terms. The results indicated that phenotypic-genotypic associations can established for ASD by reducing its genotypic heterogeneity i.e. clustering of functionally similar genes. However, to associate these clusters with phenotype, further efforts are required to enrich HPO resource. (Grant reference: PTDC/CCI-BIO/28685/2017)

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P09.027B

Resolving effects of *CASK* mutations in children with neurodevelopmental disorders

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Introduction: Mutations in the *CASK* gene cause a range of childhood neurodevelopmental disorders such as microcephaly with pontine and cerebellar hypoplasia (MICPCH), epilepsy, developmental delay and autism. *CASK*, located on Xp11.4, plays a role in neuronal differentiation and synapse function. The molecular consequences of *CASK* mutations have not been studied in human neurons. Our project aims to elucidate the downstream effects of different *CASK* mutations using patient-derived induced pluripotent stem cells (iPSCs).

Materials and Methods: Skin cells from two patients, one female with severe MICPCH and one male with autism, with different CASK mutations were programmed to iPSCs and further differentiated to functional neurons. Bulk and single-cell RNA-sequencing was performed to identify molecular phenotypes and guide morphological and functional assessment of neuronal pathology in comparison with control iPSCs.

Results: A splice-site mutation in the ASD patient decreases wild-type CASK mRNA and a tandem duplication of two *CASK* exons is expressed in the MICPCH patient. The mutations reduce CASK protein levels in differentiating neurons. Transcriptome analysis revealed dysregulation of genes involved in the synaptic vesicle cycle and single-cell RNA-sequencing indicated an imbalance in excitatory-inhibitory neuronal populations. Consequently, we study synapse morphology and spontaneous firing rates of excitatory and inhibitory neurons. Moreover, we observed neuron subtype-specific upregulation of WNT signaling pathway components.

Conclusions: We provide strong evidence that *CASK* mutations lead to perturbed neurotransmission through dysregulation of synapse vesicle trafficking and differential distribution of neuronal populations. Our results can guide drug development and aid in understanding the pathological spectrum of *CASK* mutations.

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P09.029D

Implicating genetic risk variants for circadian rhythm and sleep trait difficulties in individuals with autism spectrum disorder

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Introduction: Autism spectrum disorder (ASD) is a largely hereditary neurodevelopmental disorder characterized by difficulties in social interaction and communication, and restricted and repetitive behaviour. Sleep is disturbed in up to 80% of affected youths with ASD. Genes underlying the circadian rhythm are proposed to elucidate sleep and other timing problems in individuals with ASD. Our first aim investigates whether copy-number variants (CNVs) encompassing core circadian clock genes, circadian pathway genes, and sleep trait candidate genes, detected from previous large genome-wide association studies, are

significantly overrepresented in ASD individuals compared to their unaffected siblings.

Material and Methods: We used microarray data from probands with ASD (n=2926) and their unaffected siblings (n=2434) from the Simons Simplex Collection (SSC). CNVs were called, validated, and subsequently filtered using established pipelines and detection algorithms (PennCNV, QuantiSNP, SnipPeep).

Results: Fisher's exact test reveal that CNVs encompassed in core circadian clock genes, circadian pathway genes, and sleep trait candidate genes are significantly overrepresented in probands with ASD in comparison to their unaffected siblings (p=0.003; Odds ratio 1.6; CI95% [1.2 ; 2.3]).

Conclusions: These preliminary results are the first to implicate a broad network of sleep-related genes in the etiology of ASD. Using whole-exome sequencing (WES) data from the SSC, future investigations will evaluate the representation of probands' SNPs within sleep-related genes in comparison to their unaffected siblings. Furthermore, SNP-set Kernel Association Test (SKAT) using probands' WES and corresponding phenotypic sleep observations will identify candidate SNPs that associate significantly with disturbed sleep traits in individuals with ASD.

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P09.030A

Heterozygous *de novo* truncating mutation of nucleolin in an ASD individual disrupts its nucleolar localization

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Introduction: Nucleolin is a major nucleolar protein, playing a critical role in multiple processes, including ribosome assembly and maturation, chromatin decondensation, and pre-rRNA transcription. Due to its diverse functions, nucleolin's role has been frequently implicated in pathological processes, including cancer and viral infection. Here, we identified a *de novo* frameshifting indel mutation, p.Gly664Glufs*70 through whole exome sequencing of an autism spectrum disorder trio..

Materials and Methods: We transfected mammalian cells with GFP-tagged constructs encoding either A. wild type nucleolin; B. mutant nucleolin with the same C-terminal sequence predicted for the autism proband; C. truncation at Gly664. We co-stained with nucleophosmin antibodies to show co-localization. Real time fluorescent recovery after photobleaching (FRAP) was used to study effects on kinetics/binding dynamics.

Results: we have shown that mutant nucleolin mislocalizes from the nucleolus to the nucleoplasm. Moreover, a construct with a nonsense mutation at the same residue, p. Gly664*, shows a very similar effect on the location of the NCL protein, thus confirming the presence of the nucleolar location signal of NCL protein in this region. FRAP shows significant changes in the kinetics and mobility of mutant NCL protein in the nucleoplasm.

Conclusions: Several other studies have also reported *de novo* mutations in NCL in ASD or neurodevelopmental disorders. Altered mislocalization and dynamics of mutant NCL (p.G664Glufs*70/ p.G664*) may have relevance to the etiopathlogy of NCL-related neurodevelopmental phenotypes.

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P09.031B

Screening of ataxia samples reveals potentially pathogenic known and novel variants in *ATM* and *SACS* and likely to reveal novel genes and variants associated with autosomal recessive ataxia

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Introduction: Autosomal recessive ataxias (ARAs) represent a heterogeneous group of rare neurological disorders involving both the central and peripheral nervous system encountered in various ethnic groups. Here we report patients from 14 unrelated consanguineous families with ARA.

Materials and Methods: Patients were thoroughly examined by neurologists, radiologist, and evaluated and referred to gene testing by medical geneticists. Diagnostic targeted gene panels as well as whole exome sequencing (WES) coupled with autozygosity mapping were

performed. Supplementary or confirmatory Sanger sequencing was done together with family segregation analysis. Brain magnetic resonance imaging (MRI) were taken as part of radiological exam.

Results: Among the affected individuals, eight patients were diagnosed with carrying two separate mutations (a reported deletion and a novel compound heterozygous) in *ATM*. Affecteds from Family 2, 3, and 4 harbor a deletion in *ATM* assumingly a founder mutation with an estimated age of ~879 years. Among the cohort two families were found to carry *SACS* variants. One was already reported as a mutation and the other one is novel and likely to be pathogenic. Rest of the families were negative for any known ataxia related genes and variants.

Conclusions: Our iterative filtering of WES revealed several novel candidate genes in these families. Our study is likely to discover previously unknown ARA genes. This study was funded by the grant (No. 14-MED2007-20 to N. K.) from National Plan for Science, Technology and Innovation program (NSTIP/KACST).

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P09.032C

Novel mutation causing AUTS2 síndrome. A de novo small deletion affecting the transcription start site of AUTS2 short isoform

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Introduction: The Spanish Undiagnosed Rare Diseases Program (SpainUDP) performs extended genetic analyses and deep phenotyping to get a definite diagnosis in rare syndrome cases. We present the case of a 3-year-old male with severe generalized hypotonia and muscle weakness together with neurodevelopmental delay. He presents microcephaly and dysmorphic features highlighting micro-retrognathy, low-set ears and hypertelorism.

Materials and Methods: Trio-based exome analysis was carried out by using Nextera Truseq Rapid Enrichment kit and sequenced in NextSeq500 (Illumina).

Results: The exome analysis revealed a novel mutation consisting on a small *de novo* 30-bp deletion in exon 9 of AUTS2 gene (Autism susceptibility candidate 2, OMIM:607270), which allowed the diagnosis of this patient as AUTS2 Syndrome or Mental Retardation, autosomal dominant 26 (OMIM:615834). AUTS2 gene is involved in the development of neurological disorders. Alterations of AUTS2 gene were described as a result of genomic rearrangements, although large copy number variations and intragenic deletions or mutations have also been described to cause this syndrome. It is noteworthy that the deletion found in this patient included the transcription start site of the short isoform of the gene, which plays an important role in brain development. The gene expression analysis showed that the deletion causes reduction in the level of expression not only of the short isoform but also of the complete transcript of the gene.

Conclusions: This case helps in establishing the genotype-phenotype correlation adding more evidence to the role of mutations specifically affecting the short isoform for the development of a severe phenotype.

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P09.033D

Comprehensive genetic analysis of whole genome sequencing data from 108 individuals of 8 multigenerational spanish families affected with bipolar disorder

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Bipolar Disorder (BD) is a genetically complex neuropsychiatric disorder with an estimated heritability of approximately 70%. GWAS have shown that common risk factors substantially contribute to the development of BD and explain 25-38% of the phenotypic variance. Rare variants with higher penetrance might explain some of the hidden heritability. A research strategy to identify such rare variants is Whole Genome Sequencing (WGS) of promising multigenerational families with BD. In the present study we conducted WGS of 108 individuals in a set of 8 extended multigenerational and multiply affected families of Spanish origin.

WGS was performed PCR free at 30X. VCFs were created based on GATK´s best practice guidelines. We set up a WGS analysis workflow based on vcfR. We conducted a gene set/pathway analysis for the resulting genes using IPA and ConsensusPathDB. In addition to that we conducted Polygenic Risk Score analysis in the subset of the 8 families.

In our analysis we focused on rare variants with a minor allele frequency below 1% (ExAC database). Our first analysis is an "extended exome" analysis of WGS data. We applied a narrow analysis model to identify overlapping, rare, co-segregating variants in the affected individuals in one family which are not shared by healthy individuals within the same family. Additionally we applied an analysis model for incomplete penetrance. In total we identified 3 protein truncating and 9 missense variants. PRS analyses in the families show very diverse risk profiles for healthy and affected individuals. Gene set enrichment analyses mainly highlight immunological genes.

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Bi-allelic MYORG variant carriers exhibit primary brain calcification with a distinct phenotype

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Introduction: Primary Familial Brain Calcification (PFBC) is a rare disorder with diverse neuropsychiatric expression. Mutations in four genes cause autosomal dominant PFBC (AD-PFBC). Recently, bi-allelic *MYORG* pathogenic variants have been reported to cause autosomal recessive PFBC.

Methods: We screened *MYORG* by whole exome sequencing in 29 unrelated probands negatively screened for the AD-PFBC genes, studied the clinical and radiological features in bi-allelic pathogenic variant carriers and compared them to that of 102 AD-PFBC patients.

Results: We identified 16 patients from 11 families with a bi-allelic rare or novel predicted damaging variant. *MYORG* patients exhibited a high clinical penetrance (median age of onset of 52 years, range: 21-62) with motor impairment at the forefront. Dysarthria was the presenting sign in 11/16 patients. In contrast to AD-PFBC patients, 80% symptomatic patients eventually presented at least 4 of the following 5 symptoms: dysarthria, cerebellar syndrome, gait disorder, akinetic-hypertonic syndrome and pyramidal signs. In addition, *MYORG* patients exhibited the most severe pattern of calcifications. Strikingly, 12/15 presented brainstem calcifications in addition to extensive calcifications in other brain areas (basal ganglia, cerebellum ± cortex). Among them, 8 patients exhibited pontine calcifications, which were never observed in AD-PDFC. Finally, all patients exhibited cerebellar atrophy as confirmed by MRI Voxel Based Morphometry. Of note, in three families, the father carried small pallido-dentate calcifications, suggesting a putative phenotypic expression in some heterozygous carriers.

Conclusions: We confirm *MYORG* as a novel major PFBC causative gene and identify a recognizable clinico-radiological phenotype in the interface between PFBC and spino-cerebellar ataxias.

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Identifying novel *KIF1C*-cerebellar ataxia variants by exome sequencing and molecular characterization

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Hereditary cerebellar ataxias are a group of neurodegenerative disorders with a high clinical and genetic heterogeneity, characterized by incoordination of movement and speech, and unsteady gait. Several genes have been identified as causative of these diseases; however, several families remain without molecular diagnosis.

In this study, we used homozygosity mapping and exome sequencing to study a cohort of Portuguese families with autosomal recessive cerebellar ataxia, identified during a systematic population-based survey. These analyses revealed two novel homozygous variants in *KIF1C* in two families with spastic ataxia: a frameshift variant (c.393_396del, p.Ser131Argfs*13) and a splice site variant (c.1166-2A>G), both segregating with the disease and confirmed by Sanger sequencing. Western blot analysis

showed that the frameshift variant, located in the motor domain, leads to a truncated protein that lacks most of the KIF1C functional domains. We also demonstrated, using a minigene assay, that the splice variant abolishes the acceptor splice site, activating a new one 8 bp downstream. This change is predicted to cause a frameshift and a premature stop codon, thus leading to a truncated protein (p. Gly389Aspfs*27).

KIF1C encodes a member of the kinesin-3 family, which are microtubule-based motor proteins. Variants in *KIF1C* have been previously associated with SPG58 and SPAX2, both autosomal-recessive diseases combining spasticity and cerebellar signs. Our study revealed two novel *KIF1C* variants in two families with a phenotype compatible with SPAX2. These variants probably cause KIF1C loss-of-function, supporting the role of intracellular trafficking in the pathogenesis of cerebellar ataxia.

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P09.038A

In-depth genomic investigation reveals a wide spectrum of deleterious variants in 120 cases of idiopathic cerebral palsy

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Cerebral palsy (CP) is a group of neurodevelopmental disorders affecting movement and posture, in which the role of genetic variation is yet to be fully established. Here we perform comprehensive genetic analysis on a cohort of 120 idiopathic CP families by integrating 35X whole-genome sequencing, 500X whole-exome sequencing, 5000X mitochondrial genome sequencing, and high-density cytogenetic microarray. We find that up to 40% of idiopathic CP cases are attributed to de novo and inherited deleterious variants in a spectrum of genes, including novel candidate genes such as *TYW1* and *GPAM*, the dysfunctional copies of which are shown to cause abnormal neurodevelopment in organismal models.

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P09.039B

TORCH-like encephalopathy due to de novo COL4A1 mutation

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Cerebrovascular destructive lesions of the brain are well-known antenatal manifestations of COL4A1 pathogenic variants. These lesions include both limited porencephales and wider lesions such as hydranencephaly and schizencephaly, sometimes associated with intracranial calcification. Nature of the lesions depends on timing and anatomical extension of the vascular insufficiency.

We report the case of a girl born in Kazakhstan after prenatal suspicion of microcephaly. She received intensive care after birth and showed infantile spasms at 3 months. At our first examination at 9 months, she presented severe development delay, tetrapyramidal involvement, marked microcephaly and epilepsy. At 11 years, the girl was profoundly intellectually disabled and showed stable microcephaly at -7 SD. Initial brain imaging showed widespread ulegyria, incomplete gyration with major widening of the Sylvian fissure and polymicrogyria, as well as band-like calcifications. This suggested a TORCH infection.

Analysis of the OCLN gene, responsible for Pseudo-TORCH syndrome 1, revealed no variation. Cytomegalovirus fetopathy was suspected since the virus was found in the urine. Finally, targeted sequencing of COL4A1 revealed the de novo heterozygous p.Gly148Arg variant, typically altering a Gly-X-Y motive of the collagen triple helix.

COL4A1 mutations should be considered in unproven TORCH-like encephalopathies combining widespread vascular insufficiency of the fetal brain, gyration abnormalities and calcifications.

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P09.040C

Development of a cellular model to study the role of CR1 in Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative dementia in the elderly, affecting between 5 and 10% of the population over 65 years of age, and increasing its prevalence with age. The ratio of isoforms F and S of CR1 (CD35), determined by the presence of some haplotypes or variants, has been related to late onset AD. Particularly the significant presence of the long isoform (S) is related to a lower total amount of CR1, which leads to a worse elimination of the β-amyloid peptide (Aβ), greater activation of the microglia as an inflammatory response, and neuronal death.

In this work we have generated a mixed cellular model of neuroinflammation, using as a model the cell lines SH-SY5Y (neuron), 1321N1 (astrocyte) and HCM3 (microglia). The results shown that these co-cultures are able to activate the glial cells when treated with lipopolysaccharide toxin (LPS) or Aβ, increasing the protein markers for each activated cell type. This cellular model also increases the expression of the genes of the complement complex proteins (*CR1*, *C1q*, *C3* and *C4*) in response to these treatments. Preliminary results also suggest that the silencing of CR1 in this model decreases neuronal viability with Aβ, pointing to the role of this protein in neuronal survival in AD.

This model could be a useful tool to test the effect of gene or pharmacological therapies to improve the response to Aβ and neuroinflammation, thus improving neuronal survival in AD.

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P09.041D

Single cell sequencing of iPSC neural cells from Down syndrome patients uncovers perturbed cell differentiation

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Background: Down syndrome (DS) is a leading genetic cause of intellectual disability and approximately 95% of patients with DS have a full trisomy 21 (T21). This result in a global transcriptional dysregulation and studies suggest an altered distribution of neuronal and glial cells in DS brains.

Methods: We established induced pluripotent stem cells (iPSC) from a patient with full T21 and a healthy control individual. The iPSCs were differentiated into neural cells for 30 days using an undirected protocol. Libraries was prepared using the Chromium Single Cell 3'Reagent Kits v2 (10X Genomics), sequenced on a HiSeq 2500 PE (Illumina) and analyzed using CellRanger V2.0 (10X Genomics) and Monocle 2 (Trapnell Lab).

Results: Cluster analysis from euploid cells shows that 63% correspond to radial glia, 6% to intermediate neuronal progenitor cells (nIPC), 20% to neurons and 5% to vascular and leptomeningeal cells (VLMCs). In contrast, the cluster analysis of T21 cells shows that 24% correspond to radial glia, 5% nIPCs and only 4% neurons. Importantly, 56% of trisomic cells correspond to VLMCs, making this cluster predominant.

Conclusion: In our model of T21 neurogenesis we observe a reduced proportion of neuronal cells and an increased proportion of glia cell. The formation of the VLMC population in iPSC neural cells with T21 appears to be continuous and with a differentiation trajectory distinct from that of euploid cells consistent with DS brain development.

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P09.042A

Rare complex chromosome mosaic with two different chromosome-15 derived supernumerary marker chromosomes in a boy with developmental delay and behavioural abnormalities

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Case report: We report on 5 years old boy with global developmental delay, short attention span, lack of cooperation, sleep disturbance, and microcephaly. He is the only child of parents of Nepalese descent.

Materials and Methods: Conventional karyotyping was performed using standard GTG banding technique. FISH analyses were performed on metaphase spreads according to the manufacturer's specifications. For microarray analysis, CytoSure Constitutional 180K microarray was used.

Results: Chromosome analysis revealed four different cell lines: cell line I (28 %): normal karyotype; cell line II: bisatellited supernumerary marker chromosome (SMC) (50 %); cell line III: a different SMC (3 %); cell line IV: both SMC's (19 %). The combined use of FISH probes and microarray analysis led to the following characterizations: the bisatellited marker chromosome as monocentric chromosome 15 with no clue for euchromatin; the second marker chromosome as a complex ring chromosome 15 with a 9,8 Mb duplication 15q11.2q13.3 including the Prader-Willi-/Angelman syndrome critical region, together with a 538 kb gain in band 15q11.2 (Burnside-Butler syndrome region) presumably in triplicated form.

Conclusion: We present a patient with a partial pentasomy 15q11.2/tetrasomy 15q11.2q13.3 mosaicism. Patients with the recurrent chromosome 15q11-q13 duplication syndrome are characterized by intellectual disability, seizures, and behavioural abnormalities, while patients with a triplication 15q11-q13 have a more severe neurosychological phenotype. Due to the mosaicism the further development of our patient is hardly to predict making genetic counseling a difficult task. Besides, none of the modern NGS-based techniques is able to solve this case correctly.

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P09.043B

Gene expression analysis in cortical neurons differentiated from 32 induced pluripotent stem cell (iPSC) lines of *THAP1* mutation carriers and controls

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Introduction: Dystonia is a heterogeneous movement disorder and can be caused by mutations in *THAP1* (DYT-THAP1). However, only ~50% of *THAP1* mutation carriers develop dystonia. The underlying mechanisms of the reduced penetrance remain elusive. Maturing neurons generated from induced pluripotent stem cells (iPSCs) could serve as an *in-vitro* model to unravel penetrance-relevant mechanisms.

Material and Methods: Human dermal fibroblasts were reprogrammed using non-integrating Sendai virus and iPSCs were cultivated on Matrigel with E8 and mTeSR media. Neural differentiation of 32 iPSC lines into cortical neurons was achieved using dual SMAD inhibition, two neural rosette stages, and a dissociation step. Cortical neurons were harvested on day 44 of differentiation for gene expression analysis by quantitative PCR.

Results: In the 44-day old neuronal lines, *THAP1* expression was elevated in mutation carriers with p. Lys158Asnfs*23 and p.Ser21Cys mutations, but not in p. Arg13His carriers. A trend of increased *THAP1* expression in affected compared to unaffected carriers was observed. Expression of *TOR1A*, another dystonia gene that possibly is regulated by the transcription factor THAP1, seemed unaltered. Furthermore, *THAP1* expression was independent of a seemingly penetrance-linked polymorphism in the *KAT6A* promotor, a region that loops to the *THAP1* promotor as shown by 4C analysis.

Conclusion: We observed higher expression of *THAP1* in some mutation carriers compared to controls depending on the mutation, which may be explained by the autoregulation of *THAP1*. Further, the *THAP1* expression level might correlate with the disease status. Future transcriptomic profiling will shed light on dysregulated signaling pathways resulting in DYT-THAP1 dystonia (FOR2488).

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P09.044C

Diagnostic yield and clinical relevance of exome trio analysis in patients with epilepsy

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Introduction: Epilepsy is a chronic disorder characterized by recurrent unprovoked seizures. Frequently, these patients have different type of seizure and may have other neurological symptoms. The goal of this study was to

identify the genetic causes and to establish the diagnostic yield of trio analysis on patients with isolated seizures or with syndromic clinical presentations.

Patients and Methods: A cohort of 81 probands was sequenced by Trio whole exome sequencing. The median age was 7 years old. In 78% of the probands, seizures were described as part of a more complex phenotype. Genomic libraries were generated with Ion AmpliSeq™or SureSelect^{XT}and sequenced by Ion Proton™/ S5™XL or NovaSeq 6000. The data were processed using an "in house" developed pipeline.

Results: A genetic diagnosis was obtained on 28 out of 81 patients, leading to a diagnostic yield of 35%. Among the identified variants the distribution was, 64% de novo, 18% homozygous, and 14% hemizygous, with a large proportion of missense variants (64%). Diagnostic yield of 42%, was obtained in the syndromic cases (n=63), identifying variants not previously reported in targeted analysis. In the cases with isolated seizures (n=22), the genetic diagnosis was only established in two cases, through the identification of de novo variants in the genes *COL4A3BP* and *TBR1*, previously associated to a more complex phenotype.

Conclusions: Exome trio sequencing is a cost-effective strategy with a high diagnostic yield in syndromic patients with epilepsy that enables a phenotypic broad-spectrum diagnosis. This approach contributes significantly to the identification of new candidate genes.

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P09.045D
CNVs in patients with epilepsy - Czech experience

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Epilepsy is heterogeneous chronic neurological disorder with prevalence 8/1000. Copy number variants (CNVs) are responsible for 2-11 % of epilepsies. We present cohort of 193 patients with epilepsy, both children and adults, divided in two groups - 49 patients with isolated epilepsy and 144 patients with syndromic epilepsy (i.e. associated with developmental delay, dysmorphic features etc). ArrayCGH platform SurePrint G3 ISCA 4x180 and 8x60 (Agilent Technologies) were used for the examinations. We detected CNVs in 32/193 of patients (16.6 %): 16 deletions (50 %), 12 duplications (38 %), one triplication (3 %) and more than one CNV were detected in three patients (9 %). 15 CNVs were pathogenic and explaining the phenotype, three CNVs were probably pathogenic, two were benign and 12 were variants of unknown significance (VOUS). Pathogenic and probably pathogenic CNVs were detected only in syndromic patients. In patients with isolated epilepsy only VOUS and benign variants were detected. Origin of variants was determined as maternal (20 %), paternal (13 %), *de novo* (30 %) and was unknown in 37 % of cases. CNVs size ranged from 15 kb to 9 Mb. Except of CNVs located in regions associated with known syndromes and epilepsy "hotspots", we detected new non-recurrent CNVs. Using arrayCGH, we identified CNVs as a cause of epilepsy in 18/144 syndromic patients (12.5 %). CNVs play an important role in aetiology of epilepsies. Therefore arrayCGH should be included into diagnostic algorithm as a first-choice genetic method in patients with syndromic epilepsy. Supported by: 00064203, NF-CZ11-PDP-3-003-2014, 17-29423A

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How far can we go? Whole genome sequencing, periodic reanalysis and international collaborations expands our understanding of the causes of developmental and epileptic encephalopathy

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Introduction: There is a pressing clinical need to determine the molecular causes of DEE to guide management and genetic counselling. Our trio exome sequencing (ES) study (2016-2017) had a high diagnostic yield (15/30: 50%), demonstrated cost-effectiveness and delineated novel genetic causes of DEE; but could we improve the diagnostic yield further?

Materials and Methods: Trio WGS (Illumina HiSeqX platform) was applied to 15 individuals undiagnosed by our ES study and 16 individuals undiagnosed by massively-parallel sequencing panel. In-house platforms Seave, Mity, ClinSV, Introme and ROHmer facilitated variant annotation and filtration and identification of regions of homozygosity and mitochondrial, non-coding and structural variants. Collaborations were initiated to clarify pathogenicity of novel variants.

Results: 8 additional diagnoses were made in the 15 individuals from the ES study: 5 were detectable by ES but required collaborative clinical/functional studies to prove pathogenicity; 3 (20%) were genomic mechanisms undetectable by ES (complex structural variants). 11 diagnoses were made in the 16 panel-negative individuals (68%): the majority (n=10) in genes excluded from the panel, 1 was missed for technical reasons.

Conclusions: An ES or WGS approach plus periodic reanalysis and international collaborations can result in a cumulative diagnostic yield of over 75% for DEE. WGS can robustly detect structural variants; ongoing research will assist non-coding variant interpretation. Individuals undiagnosed after intensive genomic analysis often had normal development prior to seizure onset, and lacked congenital anomalies and neurological progression: they may have alternative causes such as somatic mosaicism or polygenic variants. **Funding:** NHMRC, NSW Health, Kinghorn Foundation

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Multi-omic approaches implicate dysregulated calcium pathways in essential tremor

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Introduction: The genetic etiology of one of the most common movement disorders, essential tremor (ET), is not yet fully characterized. Currently, studies have focused on investigating common or rare genetic variants but have yet to investigate ET transcriptomes.

Methods: To identify relevant pathways and genes, the transcriptome of two cerebellar regions (dentate nucleus and cortex) derived from 16 cases and 16 age- and sex-matched controls was interrogated by RNA sequencing. A genome-wide gene association study (GWGAS) was conducted using 1,778 cases and 5,376 control individuals. Afterward, pathway enrichment was done using genes shown to be significant after Bonferroni correction (P<2.7E-06) to narrow down transcriptomic pathways.

Results: We identified several novel dysregulated genes including *CACNA1A*, a calcium voltage-gated channel implicated in ataxia and reported to cause tremor in *Cacna1a*-knockout mice. Furthermore, several pathways including axon guidance and calcium channel activity were significantly enriched. The GWGAS also revealed a significant enrichment across genes associated with calcium ion-regulated exocytosis of neurotransmitter. Finally, the dentate nucleus and cerebellar cortex appeared to have different transcriptomes, supporting the notion of spatially different transcriptomes across the cerebellum.

Conclusions: The identification of dysregulated pathways in post-mortem cerebellum from patients can increases our understanding of the pathogenesis for this understudied condition, which may reveal potential targets for therapeutic treatment. Calcium-related pathways were shown to be relevant to ET and future studies should investigate the underlying mechanisms of this disruption. We thank the participants for providing DNA and the Canadian Institutes of Health Research (#RN254517-332736).

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Study of TTR familial amyloid polyneuropathy in Greek patients

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Introduction: Familial amyloid polyneuropathy (FAP) is a rare, autosomal dominant, neurodegenerative disorder, usually associated with mutations in the transthyretin gene (TTR). The most common mutation worldwide is p. Val30Met. The present study aims to identify TTR gene mutations in Greek patients and attempt genotype-phenotype correlations.

Materials and Methods: We studied 16 Greek individuals belonging to 10 families. These included 8 patients referred for suspected FAP, 6 asymptomatic relatives of patients, and 2 patients with an FAP clinical phenotype that were, however, originally referred for Charcot-Marie-Tooth (CMT) gene testing and selected from a group of 800 suspected CMT patients, negative for CMT1A. The molecular diagnosis included screening for p.Val30Met with restriction fragment length polymorphism analysis (RFLP) and Sanger sequencing of the entire coding region of the TTR gene.

Results: The p.Val30Met mutation was detected in 3 out of 10 index-cases (30%). In the remaining probands, Sanger sequencing identified the p.Cys10Arg, p.Arg34Gly, p. Arg54Thr, p.Ala81Thr, p.Glu89Gln, p.Glu89Lys and p. Val94Ala mutations. The p.Val30Met cases had the typical early-onset neuropathic phenotype, without early cardiomyopathy. The remaining patients had later onset neuropathy and concomitant cardiac amyloidosis. In two of these patients the presenting symptom was exertional dyspnea.

Conclusions: The p.Val30Met mutation is a frequent but not universal cause of FAP in the Greek population. It seems to present with the early-onset classic phenotype. Other mutations exhibit more varied phenotypes with later onset. Interestingly, some FAP patients are hidden within the large group of "suspected CMT".

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Investigation of the role of *GRN* upstream open reading frames in frontotemporal dementia

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Introduction: Frontotemporal dementia (FTD) is the second most common subtype of dementia regarding people under 65 years old. It is featured by degeneration of the frontal and temporal lobes of the brain and leads to behavioral and language impairments. Upstream open reading frames (uORFs) play an important role in controlling protein expression. Variants in these elements have been linked with certain diseases. In this project, we will investigate the role of uORFs in granulin (*GRN*)-associated FTD.

Materials and Methods: Targeted resequencing of uORF-harboring regions of *GRN* by design of an amplicon target amplification assay (Agilent, https://www.agilent.com) was performed. Screening was done in the Belgian cohort, consisting of 389 patients and 415 control individuals.

Results: Investigation of ribosome profiling databases and literature allowed us to identify four uORFs for *GRN*. Preliminary screening in the Belgian cohort revealed 2 variants present within a *GRN* uORF. First variant (2/415 controls, 1/389 patients) is within a splice region and its presence could promote differential splicing by the spliceosome machinery. The second variant (3/415 controls, 2/389 patients) leads to a change in the amino acid sequence of the uORF close to the termination codon, which could influence ribosomal occupancy, thus affecting translations of the downstream protein.

Conclusions: These variants could act as potential modifiers, affecting GRN serum levels or age at onset. Therefore, functional validation of these variants could lead to the development of biomarkers and provide novel mechanistic insights in FTD.

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bdetection of gene dosage alterations in patients with spinocerebellar disease using an exon-dense snp array

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AIMS: In spite of NGS many patients remain without a diagnosis. In some cases this might be due to undetected pathogenic copy number variants. We evaluated a high-density SNP-array to assess dosage abnormalities in ataxia and spastic paraplegia.

Methods: 10 patients were genotyped with CytoScan_-XON_array (ThermoFisher). The following deletions had been previously detected by MLPA: *SPAST* exon 1 (1.52kb, N=2), exons 6-7 (0.58kb, N=2) and exons 2-17 (154.4kb, N=1); *SPG11* exon 29 (0.21kb, N=1); *ABCD1* exons 3-10 (10.11kb, N=4). We included 94 control samples from the 1000G_IBS collection. Patients with deletion of *SPAST* exon 1 and *SPG11* exon 29 were genotypes in duplicate and triplicate, respectively, to evaluate inter-experiment reproducibility. Data analysis was carried out with CHAS® software.

Results: All assays passed quality check and were consistent between replications. Concordant results from MLPA and array were obtained in 5/10 cases: *ABCD1* exons 3-10, *SPAST* exons 1, 6-7, and 2-17, the latter including contiguous genes (*SLC30A6*, *NLRC4*). In 3 patients *ABCD1* deletion was partially detected, exons 3-5 (3.51kb). The deletion was not detected by the array in 2/10 cases: *SPAST* exons 6-7, *SPG11* exon 29, albeit observable upon visual examination in all 13 assays. There were no gains or losses in control samples.

Conclusions: CytoScan_XON array is useful to identify gene deletions in parallel, even at exon level. False negative rate was 20% (2/10), however these deletions were visually observable. Factors influencing the efficiency of the approach include the size of the deletion and presence of pseudogenes. Funding: ISCIII (PS09/01830; PI17/01582)

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P09.053D

Autosomal recessive spinocerebellar ataxia with first homozygous duplication in GRID2 and review of the literature

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Introduction: Autosomal recessive cerebellar ataxias are characterized by abnormal structure of the cerebellum and spinal cord. GRID2 is involved in ataxia through both loss-of-function mutations and gain-of-function mutations due to respective deletions and missense variations. Here, we report the identification and characterisation of a novel ARCA caused by bi-allelic duplication of GRID2 in two siblings.

Methods: Illuminia® Truesight One clinical exome was performed on one of the siblings with MiSeq next-generation sequencing platform. Whereas chromosomal microarray analysis was performed on the entire family by using Affimetrix Optima® chips. Variants were evaluated based on the phenotype and standard in silico tools.

Results: No disease causing mutations were reported as a result of the clinical exome test.Chromosomal microarray analysis showed a ~121 kb homozygous duplication of GRID2 (chr4:94,447,511-94,569,021), including exon 14, in two siblings.

Conclusion: Previously there has been reports of homozygous deletions and missense mutations of GRID2, but our study is the first one to identify a homozygous duplication of the gene. This mutation, which segregated in two siblings with autosomal recessive spinocerebellar ataxia, will help us understand the effect of the exon 14 of GRID2 on the phenotype and the structure of the GluRD2 protein. These findings provide us with the conclusion that chromosomal microarray should be the first step of the diagnostic process of autosomal recessive ataxia types.

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Cadherin 13 interacts with Integrin Beta-1 and Integrin Beta-3 to regulate inhibitory synaptic function in a human neuronal model

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The brain is a complex neural network that requires a precisely tuned interplay of excitatory and inhibitory signals in order to function. Disruptions in synaptic connectivity, in particular the balance between excitation (E) and inhibition (I), have been shown to underlie neurodevelopmental disorders (NDDs). However, the mechanisms underlying this coordination remain elusive. One gene that is implicated NDDs is *Cadherin 13* (*CDH13*). Dysregulations of *CDH13* have been linked to attention-deficit/hyperactivity disorder and comorbid disorders such as autism and schizophrenia. Studies in mice showed that Cdh13 localises at the inhibitory presynapse, and when knocked out leads to increased inhibitory drive onto hippocampal CA1 pyramidal neurons. However, the mechanism by which CDH13 regulates the function of inhibitory synapses in human neurons remains unknown. To understand the function of CDH13 in human neurons, we generated both GABAergic and Glutamatergic neurons via controlled differentiation of Induced Pluripotent Stem Cells (hIPSC). Using RNA interference we reduced CDH13 expression in GABAergic neurons. We investigated neuronal network communication by means of Micro-Electrode Arrays, whereas on a single-cell level, inhibitory synaptic transmission was assessed using whole-cell patch-clamp recordings. Our data shows that CDH13 knockdown in human GABAergic neurons increases inhibitory control on glutamatergic neurons recorded by MEA. Moreover, cell-adhesion assay revealed that ITGβ1 and ITGβ3 play an opposite role in the regulation of inhibitory synaptic strength via interaction with CDH13. In summary, these results point towards an important role for CDH13 in inhibitory synapses via CDH13-ITGβ1/ITGβ3 interaction, which could be critical in the maintenance of the E/I balance.

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A rare etiology of epileptic encephalopathy: *HECW2* mutations

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Introduction: Heterozygous mutations in the *HECW2* cause ''Neurodevelopmental Disorder with Hypotonia, Seizures, and Absent Language; NDHSAL'' (#617268), which is characterized by severe developmental delay, absent speech, epilepsy, hypotonia, dystonia/dyskinesia, microcephaly, and structural brain anomalies. Here we present a novel *de novo* heterozygous mutation in the *HECW2* gene in a patient with growth retardation and epileptic encephalopathy. Material-Methods: A seven-year-old boy who referred to our clinic with epileptic encephalopathy had severely delayed psychomotor development, absent speech and feeding difficulty. His height, weight and head circumference measurements were less than 3rd centile. His motor tonic seizures were reported to start at the age of two and to continue daily. Generalized epileptic changes were present in his EEG. Progressive atrophy of cerebrum, cerebellum and brainstem was seen in the cranial MRI. WES analysis was performed in order to elucidate the aetiology of the neurodevelopmental disorder.

Results: A novel *de novo* heterozygous mutation c.4484G>A (p.Arg1495Lys) was found in the *HECW2* gene.

Conclusion: To the best of our knowledge, only four mutations in the *HECW2* have been identified in ten patients. Because of the rareness of the reported cases, here we present the patient with a novel *HECW2* variant associating with his clinical findings. Similar reports are still needed to clarify genotype-phenotype correlations of NDHSAL.

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Dominant negative heterozygous mutation in Erlin2 prevents degradation of IP3 receptors and is responsible for hereditary spastic paraplegia 37

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Introduction: Erlin 2 is an endoplasmic reticulum protein mediating the degradation of IP3 receptors. Autosomal recessive or dominant mutations of *Erlin2* have been implicated in complex motor neuron diseases with cognitive impairment, including hereditary spastic paraplegia SPG18.

Materials and Methods: We analysed the exome sequencing of four patients of a large family with autosomal dominant spastic paraplegia significantly linked to the SPG37 locus, a chromosomal region containing the *Erlin2* gene and overlapping the SPG18 locus. Fibroblasts of an affected patient and of a sex and age-matched control were subjected to an agonist of IP3 receptors to analyse their degradation by the proteasome. Fibroblasts were also transfected with siRNAs targeting the pathological allele to observe the reversal of the phenotype.

Results: We identified the heterozygous c.194C>T (p.T65I) missense variant in *Erlin2* segregating in the family. Mutated and wild type alleles were expressed at the same level in fibroblasts. Compared to controls, IP3 receptors degradation was slower in mutated fibroblasts, suggesting a degradation process blockage. To test the hypothesis that the variant acts as a dominant negative, we specifically downregulated the p.T65I variant using siRNA. While Erlin2 levels remained normal in control lines, a 50% downregulation of the p.T65I allele was observed in the SPG37 line with reduced degradation capacities of IP3 receptors.

Conclusion: We report that a heterozygous missense variant of *Erlin2* accounts for SPG37 through a dominant negative effect on the IP3 receptors degradation *in vitro*.

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Hereditary spinocerebellar degeneration in Sudan: identification of variants in known and new genes in a large cohort

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Introduction: Hereditary spinocerebellar degeneration (SCD) encompasses a spectrum of clinically and genetically heterogeneous disorders, with phenotypes ranging from pure spastic paraplegia or pure cerebellar ataxia to more complex forms. Sudan is located in east Africa which is a region known for the high genetic diversity among its population. The Sudanese population is characterized by high rates of consanguineous marriages (40-49%).

Methodology: We used next generation sequencing targeted genes panel screening and whole exome sequencing to study 25 Sudanese families with SCD. We analyzed the sequencing data using online and in-house bioinformatic tools. We confirmed the segregation of the identified candidate variants with the disease phenotype in the studied families using Sanger sequencing.

Results: We identified 24 culprit variants, 18 of these were in 15 genes previously linked to SCD and six were located in six new candidate genes never linked to pathological phenotypes in man. Out of the 18 variants in the known SCD genes, 11 variants were novel, one was autosomal dominant, six were compound heterozygous and 11 were homozygous. Three of the variants were possible founder variants: NM_024306.4(*FA2H*):c.674T>C and NM_021222.2(*PRUNE1*):c.132+2T>C, each in two families; NM_138422.3(*ADAT3*):c.430G>A in one of our families and was previously identified in families from the gulf area.

Conclusion: We studied 25 Sudanese families with hereditary spinocerebellar degeneration, and we identified the causative mutations in 88% of these families. The 24 culprit variants were found in 15 genes previously linked to the phenotype and in six new candidate genes under validation through functional studies.

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P09.058A
Understanding the role of HNRNPU in brain development and neurodevelopmental disorders

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Background: Mutations affecting *HNRNPU* (Heterogeneous nuclear ribonucleoprotein U) located on chr1q44 have been described in individuals with various neurodevelopmental disorders. HNRNPU is one of the components of the spliceosome and shown to regulate the 3D genome organization. However, the role of HNRNPU in neuronal development has not been characterized.

Material and Methods: Induced pluripotent stem cells from controls and individuals with HNRPU mutations will be used to investigate its role in neuronal development. HNRNPU expression is measured during the early stages of the neuronal differentiation using the neuroepithelial stem cell-like (NES) model followed with detailed investigations of the 3D genome organization, transcriptomics including splicing patterns and DNA/RNA targets of HNRNPU using high-throughput methods.

Results: We have identified a monozygotic twin pair with severe ID, autism and epilepsy carrying a ~44.3Kb heterozygous deletion of *HNRNPU* and COX20. The expression of HNRNPU in NES cells was confirmed to be reduced ~50% due to the deletion as expected. In control cell lines, the *HNRNPU* expression was reduced upon neuronal differentiation, however similar pattern was not seen in the deletion carrier. We are currently investigating the early time points of neuronal differentiation and how the multiple roles of HNRNPU are connected to the typical and atypical trajectories of the neuronal development using multiple omics-methods.

Conclusions: Reduction of HNRNPU levels interfere with differentiation at an early phase of neuronal commitment. Our study will elucidate the molecular mechanisms that could underlie the NDDs associated with HNRNPU mutations and 1q44 deletions.

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P09.059B
Heterozygous mutations of *HTRA1* and cerebral small vessel diseases, a diagnostic challenge

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Heterozygous *HTRA1* missense mutations have recently been involved in an autosomal dominant Cerebral Small Vessel Disease (cSVD). Functional in vitro analysis has been performed for a very limited number of these variants, suggesting a probable dominant negative effect. The aim of this study was to investigate the implication of *HTRA1* heterozygous mutations in cSVD in a large consecutive series of patients. Samples from 2135 unrelated adult patients were referred consecutively from 2016 to 2018 in the neurovascular genetics department of Lariboisière hospital for targeted sequencing of known cSVD genes, including *HTRA1*. Heterozygous *HTRA1* missense variants with a prevalence < 1/10 000 in polymorphism databases and predicted to be pathogenic by at least 2 in silico prediction softwares were retained. Among these 2135 patients, 35 patients (1.6%) showed a heterozygous candidate missense variation. Only 7 of the detected variations were previously described (and 5 functionally tested). 16 of them predicted as being pathogenic have not yet been reported. Frequencies of candidate missense in our series and control samples from GnomAD are significantly different (p= 6.4E-7, OR: 1.8-3.7), confirming the involvement of *HTRA1* in cSVD pathogenesis. Co-segregation of these variants in affected relatives is ongoing. However, additional functional screening of these variants, including residual htra1 enzyme activity measurement and trimerization analysis are now required to establish or infirm their pathogenicity, raising a strong challenge in a diagnosis context.

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P09.060C
Genetic epidemiology analysis of Huntington and Huntington like disease among Hungarian patients

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Introduction: Huntington and Huntington-like diseases are progressive neurodegenerative disorders which are characterized by uncontrolled movements (chorea), emotional problems, and loss of thinking ability. The classical Huntington chorea is associated with the abnormal trinucleotide (CAG) repeat expansion in the huntingtin (*HTT*) gene. In the remaining cases, mutations of the *C9ORF72* hexanucleotide- (G_4C_2), *TBP* (TATA box-binding protein) trinucleotide (CAG/CAA) repeat and prion protein (*PRNP*) genes mutations may also cause a Huntington-like clinical phenotype. *Aims:* In this study the genetic background of chorea-like movement disturbances were investigated among our Hungarian cohort. *Patients and Methods:* In 375 (mean age 45.2 years) patients with choreiform movement disorder, the *HTT, C9ORF72, TBP* repeats and *PRNP* mutations were analysed.

Results: The expanded *HTT* allele (>40 CAG repeat) repeat expansion were found in 335 cases. The mean of the expanded repeat numbers is 44.6. Abnormal *C9ORF72* hexanucleotide repeats were detected in 3 cases (one patient has full mutation (>30 G_4C_2 repeat) and two others have intermedier-range (24-29 G_4C_2 repeat) hexanucleotide repeat expansions). The *TBP* repeat disease was determined in one patient, and in further one case a *PRNP* octanuclteotide in frame deletion was detected.

Discussion: The rate of occurrence of *HTT* gene abnormal trinucleotide expansion in our investigated cohorts is 90%. The remaining 10% of patients are classified as Huntington-like clinical phenotypes. In this group, the *C9ORF72* hexanucleotide gene was detected in 7.5%, while in the TBP and PRNP mutations were present in 2.5-2.5%. This study was supported by KTIA_13_NAP-A-III/6 to V. A-V. grant.

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P09.061D

A novel repeat-primed PCR assay to detect the full range of trinucleotide CAG repeats in Huntingtin gene (*HTT*)

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Accurate determination of the CAG repeat number is crucial in Huntington's disease (HD) to either confirm the diagnosis in symptomatic patients or to predict the genetic condition in subjects at risk of HD, including prenatal testing. We evaluated a novel tripled repeat primed PCR-based technology for estimation of HTT CAG repeats in 46 HD reference DNA samples, including 34 samples carrying alleles with full penetrance (> 40 CAGs). Of the samples with full penetrance alleles, 20 harbored alleles larger than 60 CAG repeats, generally seen in subjects with juvenile onset HD < 20 years and in the rarest pediatric variant. Analyzed samples had been previously tested using either flurescent repeat-flanking PCR or tripled repeat primed PCR method, or both. All samples showed full concordance with the previously verified allele sizes. Identical repeat size or sizing errors within ±1 CAG were obtained for alleles ≤42 CAG repeats, whereas sizing errors within ±3 were obtained for alleles > 43 CAG repeats, conforming to established guidelines. The assay was able to accurately size 18 samples with very large alleles comprised between 60 and 100 CAG repeats, and to detect two exceptionally large alleles with more than 200 CAG repeats. This method provides a rapid, sensitive and reliable method to accurately genotype the HTT CAG repeat stretch, and extends the detection limit of large expanded alleles to over 200 CAG repeats, thus providing a comprehensive molecular diagnostic evaluation of all HD samples, including those pediatric forms carrying extremely large, hard to detect, alleles.

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P09.062A

Psychological reactions when coping with predictive genetic testing for Huntington's disease: A qualitative study

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Introduction: Huntington's disease (HD) is an autosomal dominant disease characterized by progressive weakening

of the cognitive, psychological and motoric systems. HD may have a severe impact on family dynamics, and growing up with a parent with HD may be challenging. Adult individuals at risk can get predictive genetic testing.

Materials and Methods: We used an explorative qualitative study design and interviewed 36 persons who currently grow up or grew up with a parent with HD, and analyzed the interviews using thematic analysis.

Results: Participants described several challenges related to the decision regarding predictive testing. Some wanted to enjoy life without knowing. Others lacked motivation for getting an education and limited their involvement in romantic relationships, even though they did not know whether they had the mutation or not. Participants described ineffective coping mechanisms and a search for symptoms before predictive testing, continuing after they had tested positive for the mutation. Others, positive for the mutation, described a relief of finally having the knowledge, and an increased understanding of affected family members. Participants who had tested negative for the mutation were surprised by ambivalent feelings, and described a need for follow-up after they had received a negative test result.

Conclusion: Predictive testing for HD is a demanding process. Individuals who grow up in families affected by HD, worry about the potential genetic risk for themselves and their siblings. The current study indicates that individuals may need long-term follow-up from the healthcare system whether they test positive or negative for the mutation.

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P09.063B
The genetic background of hydrocephalus - candidate gene analysis from +6,000 exomes

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Introduction: Congenital hydrocephalus, a condition characterised by relatively excessive intracranial cerebrospinal fluid, is often considered to present neonatally. However, in practise, most patients present during infancy with slowly developing symptoms, or even later in life with subtle or neglected symptoms. Despite being a relatively common congenital disorder (1.1/1000), the genetic aetiology of hydrocephalus remains unclear.

Objectives: 1. Candidate gene analysis of 113 genes in 6,077 whole exome sequenced individuals (50 with hydrocephalus)

2. Explore the cellular pathways involving the implicated genes to understand better, how dysfunction of these genes might lead to or influence the expression of hydrocephalus.

Materials and Methods: This register-based cohort study is a sub-study of the Lundbeck Foundation Initiative for Integrative Psychiatric Research (iPSYCH) and combines data from The National Patient Registry, and The Danish Neonatal Screening Biobank. Genetic variants identified in the candidate genes were filtered based on minor allele frequency (MAF) data. We removed variants with MAF >2 x 10^{-5} in gnomAD or >5 x 10^{-3} in controls.

Results: Forty-nine mutations were identified in 31 genes. Interestingly, along with the known hydrocephalus-causing genes (*CCDC88C, MPDZ*) several of the genes implicated are involved cilia formation and function (*CEP290, USP9X, SNX10, TMEM67*) as well as corticogenesis and neuronal adhesion and migration (*CELSR2, FLNA*).

Conclusions: The results of this study suggest that a high proportion of Danish hydrocephalus patients have an underlying genetic predisposition and the genes involved affect pathways of importance for Cilia, corticogenesis as well as neuronal adhesion and migration.

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P09.065D
Evaluation of targeted sequencing as the first-tier genetic test for intellectual disability and autism spectrum disorders diagnosis

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Identification of the genetic aetiology of intellectual disability (ID) or autism spectrum disorders (ASDs) provides benefits, like specific genetic counselling, more accurate prognosis and specialized treatment. The worldwide average prevalence of ASDs is 1 in 160 children and 1-3% of ID. Due to the genetic heterogeneity of these pathologies different genetic tests are available. Today,

chromosomal microarray is the first-tier recommended diagnostic test and provides a yield around 10-15% for ID patients and around 9% for ASDs patients, depending on the cohort. Our goal is to evaluate if the diagnostic yield estimated by targeted sequencing in our cohorts of ID or ASDs subjects is higher than that obtained by CGH array. We screened 701 in-house selected ID-related genes and 189 autism-related genes in a cohort of more than 100 and 60 patients, respectively. Libraries were prepared with different capture kits and the Illumina *NextSeq500* platform was used to sequence targeted regions. Bioinformatic analysis was performed with an in-house pipeline. The diagnostic yield was 41% in the ID cohort and 20% in the ASDs cohort. Variants of uncertain significance were identified in 50% of the ID patients and 72% of the autism patients and negative results were obtained in 9% and 8% of the ID and autism individuals, respectively. ID or autism-related genes sequencing provide higher diagnostic yields than CGH array. Furthermore, copy number variants evaluation could be performed simultaneously with our pipeline. For this reason, we proposed targeted sequencing as the first-tier genetic test for ID and autism diagnosis.

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P09.066A
Development of a *LYST* deficient glutamatergic neuronal model of Chediak-Higashi Syndrome

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Chediak-Higashi Syndrome (CHS) is an autosomal recessive disorder caused by biallelic mutations in the lysosomal trafficking regulator gene (*LYST*) that results to impaired function of lysosome and lysosome-related organelles. CHS is characterized by oculocutaneous albinism, predisposition to bleeding, immunodeficiency and progressive neurological dysfunction. Bone marrow transplantation has been a symptomatic treatment for the immune defects but does not alter the course of the relentless neurodegeneration. To date, several animal models of CHS have been identified, but none of them consistently recapitulate the neurological phenotype seen in patients. Additionally, the role of LYST in lysosome biology is still is poorly understood and could be cell-dependent. For these reasons, we aim to investigate the function of LYST in a neuronal cell model and to identify targets for therapy. We used clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR interference technologies to knock-out or knock-down the expression of endogenous *LYST* in induced pluripotent stem cells (iPSCs), which were genetically engineered to facilitate an inducible expression of Neurogenin 2. With this approach, we have established a glutamatergic neuronal CHS model deficient for *LYST* expression, to analyze the localization, size and function of lysosomes in neurons. Using this technique, we were able to generate a homogenous population of neuronal cells, which are essential for global proteome, transcriptome and metabolome studies not only of CHS, but also for other Mendelian loss-of-function neurogenetic diseases.

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P09.067B
Gene-phenotype correlation of myoclonic progressive epilepsy type 3

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Progressive myoclonic epilepsies (PME) are a group of clinically and genetically heterogeneous disorders characterized by myoclonus, seizures, and neurological deterioration. PME type 3 is an autosomal recessive disease and is associated with pathogenic variants in the gene KCTD7 that encode potassium channel tetramerization domain-containing protein 7. Disruption of the function of this gene can lead to a change in the potassium ion current in the neurons of the brain. We investigate 9 patients with an average age of debut of the first attacks between 1 and 2 years of life and try to establish gene phenotype correlation. The first symptoms started after an episode of febrile fever: sudden falls without loss of consciousness, and with episodes of fading. All patients had the drug-resistant generalized myoclonic seizures. MRI of the brain, long-term video EEG monitoring, examination by various clinical specialists were performed for each patient. NGS sequencing revealed seven previously undescribed non-synonymous variants and one previously undescribed dinucleotide frame shift deletion in the last exon. Four chromosomal microarray analysis were performed for patients with large deletions suspected by NGS affecting the locus of the KCTD7 gene. Segregation analysis by Sanger sequencing were performed for all families and confirmed genotypes. Most of known likely pathogenic or pathogenic variants are located in BTB/POZ domain. However, we observed several variants located outside of this domain. It is necessary to conduct a functional analysis to confirm the pathogenic effect of the substitutions on the protein function.

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P09.068C
Genome wide association study for late-onset Alzheimer's disease in Japanese population

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By 2025, the number of people living with dementia in Japan is expected to increase to 7 million. Most common form of dementia is late-onset Alzheimer's disease (LOAD). The pathogenesis of LOAD is result from interactions among multiple environmental and genetic factors which consist of considerable hereditary components. Recent large scale genome wide association studies (GWAS) in Caucasian have revealed more than 30 genetic loci for LOAD susceptibility. In contrast, GWAS for Asian groups including Japanese are relatively modest, and have poor reproducibility of associations for identified loci in Caucasian due to possible difference of genetic architecture among ethnicity in addition to lack of study power. In this context, we launched GWAS using an ethnicity specific SNP array, Japonica. To date, we obtained the genotype data for approximately 7,000 Japanese individuals (including 2,400 LOAD, 1,400 other dementia and 3,200 controls) and imputed the data with a Japanese reference panel (ToMMo 3.5KJ) constructed using whole genome sequences from 3,500 Japanese. We tentatively conducted a GWAS using the imputed genotype data (~10,000,000 SNPs) for approximately 2,400 LOAD cases and 3,200 controls. We combined this GWAS result and the data of another Japanese GWAS reported elsewhere for meta-analysis (a total of 8,000 individuals), and found GWAS significance for the *APOE* and *SORL1* loci and a novel locus with LOAD. We further identified several new candidate susceptible loci for LOAD with suggestive significance. We also found convincing associations for several known AD loci, such as *CLU*, *PICALM* and *ABCA7* discovered in Caucasian population.

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P09.069D
Leukocyte telomere length in Huntington's Disease. A study in fully penetrant and reduced penetrant alleles

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Introduction: Huntington's Disease (HD), an autosomal dominant neurodegenerative disease, is caused by an expanded CAG repeat in the first exon of HTT gene. The disease is fully penetrant in individuals with 40 or more CAG repeats, and has reduced penetrance in the range of 36-39 repeats. Overall the age at onset, usually at midlife, inversely correlates with the number of CAG repeats, and severity of symptoms vary widely between individuals. In the present study, we investigated the relationship between Leukocyte Telomere Length (LTL) and disease progression.

Methods: LTL (T/S ratio) was measured in manifest HD patients (HD, n=62) and pre-manifest HD patients (pre-HD, n=38) with fully penetrant alleles, in subjects with reduced penetrant alleles (rp-HD, n=23), and age-matched controls (n= 76).

Results: Mean LTL values of controls, pre-HD and HD patients were significantly different ($p< 0.0001$), in the order: HD (0.58 ± 0.07) <pre-HD (0.78 ± 0.16) <controls (0.92 ± 0.09). Mean LTL values of rp-HD subjects (0.82 ± 0.16) were significantly lower than controls ($p=0.003$), but similar to pre-HD patients. An inverse relationship between mean LTL and CAG repeat number was found in the pre-HD ($p=0.03$).

Conclusion: In pre-HD patients, LTL shorten gradually according to advancing age and CAG number, up to the low values observed in HD patients. A similar LTL shortening seems to be present in rp-HD, but at more advanced age. The possible use of LTL as biomarker of disease progression is discussed.

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P09.070A
Novel variants causing inherited leukodystrophies in Sudanese families

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Background: Leukodystrophies are a group of inherited disorders primarily affecting the white matter of the CNS. There are currently 30 recognized forms of leukodystrophies with distinct clinical, biochemical and radiological characteristics. However, the genetics of these classical forms of leukodystrophies remain unknown in many parts of the world especially in Sub-Saharan Africa.

Methods: In this study, we selected 5 consanguineous leukodystrophic families from Sudan using clinical and MRI recognition pattern. Genomic DNA was extracted and screened for mutations using NGS panel testing 153 leukodystrophies and leucoencephalopathies causing genes (NextSeq500 Illumina).

Results: Three novel homozygous variants were discovered: one (c.380G>C, p.Arg127Pro) in *PSAP* gene causing MLD, and two (c.831_838DUPATATCTGT, p.Ser280Tyrfs*8 and c.971T>G, p.Ile324Ser) in *MLC1* gene in the two families with MLC. The segregation pattern was consistent with autosomal recessive inheritance. The pathogenicity of these variants was predicted using bioinformatics tools.

Conclusion: This is the first study to underlie the genetics of leukodystrophies in Sudan. Analysis of additional families are in progress in order to establish the whole spectrum of genetic variations causing inherited leukodystrophies in Sudanese families.

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P09.071B
Two associated mutations in the glutamyl-prolyl and isoleucyl -tRNA synthetases in patients with a fatal leukodystrophy

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Aminoacyl-tRNA synthetases (ARSs) have been implicated in leukodystrophies. We report a consanguineous family with two affected children. Both children presented a neurodegenerative disorder with a motor degradation and

nystagmus after 15 months, spastic quadriplegia after 3y and epilepsia after 7y leading to death at respectively 8 and 10y of age. MRI demonstrated a progressive atrophy of the spinal cord, the optic nerve, the corpus callosum and the cortex associated with a demyelinating aspect of the sustentorial periventricular WM. Axonal peripheral neuropathy was observed in both patients. Biochemichal analysis were negative

Homozygosity mapping combined to whole-exome sequencing revealed two homozygous mutations in two ARSs. One mutation in *EPRS* (c.3344C>T/p.Pro1115Leu) a bifunctional aminoacyl tRNA synthetase that catalyzes the aminoacylation of both glutamic acid and proline tRNA species, recently involved in a hypomyelinating Leukodystrophy and a second mutation in *IARS2* encodes a mitochondrial isoleucyl-tRNA synthetase reported to cause CAGSSS that is characterised by cataracts, growth hormone deficiency, sensory neuropathy, sensorineural hearing loss, and skeletal dysplasia. Co-segregation analysis confirmed that the two mutations were inherited from heterozygous carrier parents. In silico analysis predicted these variation to be deleterious. The phenotype of our patients were more severe than the reported patient with the same mutation in *ERPS* gene. Our patients had no cataract or dysplasia or growth hormone deficiency reported in *IARS2* patient. The phenotype may be the result of the mutation of the two genes. Further analysis are required to gain a better understanding of the interaction or not of the two ARSs.

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P09.072
C*KIF1A* variants are a frequent cause of autosomal dominant hereditary spastic paraplegia

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Background: Pathogenic variants in the *KIF1A* gene have been reported in autosomal recessive spastic paraplegia 30, autosomal recessive hereditary sensory neuropathy, and autosomal (*de novo*) dominant mental retardation type 9. More recently, variants in *KIF1A* have also been described in a few cases with autosomal dominant spastic paraplegia.

Methods: Clinical exome sequencing data of 315 unrelated patients with mostly 'pure' spastic paraplegia were analysed for *KIF1A* variants. Clinical characteristics of patients with *KIF1A* variants were analysed, segregation analyses were performed, and types of variants were compared.

Results: In 21 patients we identified 19 different *KIF1A* variants. Patients and affected family members showed a slowly progressive, mostly pure form of spastic paraplegia, but with a highly variable disease onset (0-57 years). Segregation analyses showed a *de novo* occurrence in five cases, and a dominant inheritance pattern in eight families. The motor domain of KIF1A seems to be the hotspot for pathogenic variants in autosomal dominant spastic paraplegia, similar to mental retardation type 9 and recessive spastic paraplegia type 30. Unlike these allelic disorders, however, dominant spastic paraplegia was also caused by loss-of-function variants outside this domain in six families. Three missense variants were identified outside the motor domain and need further characterization.

Conclusions: *KIF1A* variants are a frequent cause of autosomal dominant spastic paraplegia in our cohort (5-7%), with a high *de novo* rate. The identification of *KIF1A* loss-of-function variants suggests haploinsufficiency as a possible mechanism in autosomal dominant spastic paraplegia.

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P09.073D
Obesity-related genes and the atypical symptoms of major depressive disorder: findings from two large European epidemiological studies

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Introduction: Disentangling the heterogeneity of depression by subtyping according to clinical manifestations has been shown to be a pertinent approach to gain more insight into relationships with obesity. Indeed, previous research suggests that only the atypical MDD subtype, characterized by five symptoms (increased appetite, hypersomnia, mood reactivity, leaden paralysis and interpersonal rejection sensitivity), is associated with obesity. We investigated whether the association between atypical MDD and genetic variants related to obesity is accounted for by the appetite criterion alone or whether the other atypical criteria also contribute to this association.

Materials and Methods: Two studies, CoLaus|PsyCoLaus (Switzerland) and NESDA|NTR (Netherlands), that assessed MDD subtypes and symptoms, were meta-analysed to evaluate the associations between atypical MDD symptoms (386 and 179 participants, respectively) with the rs9939609 FTO polymorphism and the polygenic risk score (PRS) from 76 loci significantly associated with Body Mass Index according to a recent GWAS meta-analysis.

Results: Our meta-analysis shows positive associations between the symptom increased appetite and both the FTO variant and the PRS (odds ratio (OR)=1.37; 95% confidence interval (CI)=1.17-1.62, $P=1.33x10^{-04}$ and OR=1.15, 95%CI=1.03-1.29, $P=0.014$, respectively), a negative association between the FTO and the symptom leaden paralysis (OR=0.82, 95%CI=0.72-0.93, $P=0.003$) and a negative association between the PRS and the symptom rejection sensitivity (OR=0.92, 95%CI=0.85-0.99, $P=0.036$).

Conclusions: The association between increased appetite and obesity-related genetic variants drives the association between these variants and the atypical subtype, suggesting that other genes could be involved in the regulation of the other atypical symptoms, reflecting the genetic heterogeneity of MDD.

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P09.074A

Entire structure of*MECP2*cDNA expressed in a patient with Rett syndrome bearing a large deletion between the exon 4 of *MECP2* and the upstream region of *IRAK*

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Methyl-CpG- binding protein 2 (MECP2) is a responsible gene for Rett syndrome (RTT, OMIM#312750) that is inherited in an X-linked dominant manner. Pathogenic variant of *MECP2* are found in approximately 80% of patients with RTT. Partial and whole-gene deletions are accounting for 7.3% in all variants observed. We encountered a patient of RTT with large deletion of *MECP2*.

A patient is 7-year-old girl born to non-consanguineous parents at 40 weeks with birth weight of 2,484, height of 47 cm and head circumstance of 31.5 cm. Amniotic fluid karyotyping showed 46,XX. She was clinically suspected RTT based on neurodevelopmental disorder characterized by psychomotor regression with the development of distinctive hand stereotypies. However, no pathogenic variant was detected in the *MECP2* gene by direct-sequencing and MLPA.

Whole exome sequencing (WES) was performed. Mapping images of WES suggested a heterozygous large deletion between the exon 4 of *MECP2* and upstream region of *IRAK* in the patient. The deletion was verified by long-range PCR and following sequencing. Transcripts of *MECP2* were confirmed by 3'RACE analysis. The deletion was occurred in father's allele. A novel transcript derived from the deleted allele consisted of exon 3 and alternatively spliced regions of exon 4 under GT-AT rule. Coding length was expected 582 bp (194 amino acid). The translated product lacked two AT-hook domains, which are essential for DNA binding. Brain specific polyadenylation signal was found 17 bp upstream of poly A site. This work was supported by grants from the AMED.

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P09.075B

Molecular characterization of Spanish *MECP2* duplication syndrome patients: more *IRAK1* than we thought

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Introduction: *MECP2* duplication syndrome (OMIM#300260) is an X-linked neurodevelopmental disorder characterized by severe to profound intellectual disability, early infantile hypotonia, autistic traits, seizures and recurrent respiratory infections. Duplication could be *de novo* or inherited from an asymptomatic carrier mother. We have molecularly characterized 22 Spanish children with *MECP2* duplication syndrome and their carrier mothers.

Materials and Methods: Clinical characterization was done using a checklist designed for the project. Duplications were detected by MLPA and/or CGH-array and confirmed by Real-Time qPCR. Localization of the duplication was studied by FISH. *MECP2* expression was checked by RT-qPCR in blood cells and skin fibroblast cells when available. We performed XCI assay to affected females and carrier mothers.

Results: We have noticed that in the minimum duplicated region (MDR) of all our patients there are *MECP2* and *IRAK1* genes and both are overexpressed. The cohort was classified according to different chromosomal localizations of the MDR: tandem in ChrXq28, ChrXp and ChrY. The clinical severity seems to be related to the position of the duplication, where the insertion in ChrY leads to the most severe phenotype.

Conclusions: Due to the fact that *IRAK1* is present in all the duplications we hypothesize that it must be implicated in the disorder. We encourage the study of the pathways in which *IRAK1* participates. Besides, the point of insertion of the duplication seems to be crucial for the severity of the patient, as well as the genes involved in each rearrangement. Grants: Miradas que Hablan-Duplicación *MECP2* parents' association.

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P09.077D

Population incidence of congenital microcephaly in the Czech Republic

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Introduction: Microcephaly is defined as a congenital anomaly of the central nervous system which is

characterized by the decreased head circumference. The exact definition may be slightly different for different countries, in the Czech Republic the microcephaly is defined as the head circumference under the 3rd percentile for specific age and sex of the particular individual.

Methods: We present a retrospective epidemiological study of the incidence of microcephaly (ICD-10 code Q02) in the livebirths and in the prenatally diagnosed cases in the Czech Republic (time period 1994-2015). The data were obtained from the National Registry of Congenital Anomalies. During the selected time period the registry included only the cases of congenital anomalies in children under 15 years of age. Surveillance program is population based.

Results: There were 2 140 009 livebirths in the Czech Republic during 1994-2015 time period. The average incidence of microcephaly was 1.07 per 10 000 of livebirths (highest was 1.72 in 2002 and lowest was 0.33 in 1998). The anomaly was more common amongst girls - F/M ratio was 1.32/1.0. Only few cases of microcephaly were diagnosed prenatally (average incidence in prenatal diagnostics 0.12 per 10 000).

Discussion: The overall incidence of microcephaly in the Czech Republic is low - compared to the data from other population based registries. We believe, that the clinicians tend to report only severe cases of congenital microcephaly with neurological symptoms.

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P09.078A
Application of genomic tools in understanding the etiologies of microcephaly

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Introduction: Microcephaly is a genetically heterogenous condition which may be an isolated trait or in combination with other major malformations. The molecular diagnosis remains unknown in about 40% cases.

Materials and Methods: Patients with microcephaly with unknown etiology based on preliminary evaluation were enrolled. CMA was done using Affymetrix 750K. Where no pathogenic CNVs were identified, WES was done.

Results: Out of total 51 patients, CMA was done in 34 patients. Pathogenic/likely pathogenic CNVs were identified in 6 cases while candidate genes in ROH was identified in 4 cases. WES was done in 32 patients (12 consanguineous). Pathogenic/likely pathogenic SNVs were identified in 14 patients. Biallelic variations were identified in *CIT, ANKLE2, ORC1, RNASEH2A, ASPM, PEX7, CENPF, PCNT, AIMP2* and *RNU4ATAC*. Siblings with likely pathogenic variation in *CENPF* had severe short stature (-3.5SD) and microcephaly (-8SD) without ophthalmological abnormalities as reported in cases of Stromme syndrome suggesting that *CENPF* mutation can result in phenotypes of isolated microcephaly. Previously reported heterozygous pathogenic variations were identified in 3 patients (heterozygous in *MECP2 and TUBA1A* and hemizygous deletion in *PQBP1*). One patient showed double heterozygous likely pathogenic variations in *MCPH1* and *ASPM* inherited from asymptomatic parents indicating the possibility of digenic inheritance.

Conclusions: Genomic techniques gave diagnostic yield of 39% [20 out of 51]. The study identified 8 novel likely pathogenic SNVs and 4 likely pathogenic CNVs and expanded the phenotypic spectrum of *CENPF*. Pathogenic variations in X linked genes were identified in 2 cases; one with multiple affected family members. (ICMR: 63/8/2010-BMS)

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P09.079B
Genomic and phenotypic delineation of congenital microcephaly

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Background: Congenital microcephaly (CM) is an important birth defect with long term neurological sequelae. We

aimed to perform detailed phenotypic and genomic analysis of patients with Mendelian forms of CM.

Methods: Clinical phenotyping, targeted or exome sequencing, and autozygome analysis.

Results: We describe 150 patients (104 families) with 56 Mendelian forms of CM. Our data show little overlap with the genetic causes of postnatal microcephaly. We also show that a broad definition of primary microcephaly —as an autosomal recessive form of nonsyndromic CM with severe postnatal deceleration of occipitofrontal circumference—is highly sensitive but has a limited specificity. In addition, we expand the overlap between primary microcephaly and microcephalic primordial dwarfism both clinically (short stature in >52% of patients with primary microcephaly) and molecularly (e.g., we report the first instance of *CEP135*-related microcephalic primordial dwarfism). We expand the allelic and locus heterogeneity of CM by reporting 37 novel likely disease-causing variants in 27 disease genes, confirming the candidacy of *ANKLE2, YARS*, *FRMD4A*, and *THG1L*, and proposing the candidacy of *BPTF*, *MAP1B*, *CCNH*, and *PPFIBP1*.

Conclusion: Our study refines the phenotype of CM, expands its genetics heterogeneity, and informs the workup of children born with this developmental brain defect.

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P09.080C
Targeted gene panel for pediatric Movement Disorders and Cerebral Palsy

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Childhood Movement Disorders (MD), represent a heterogeneous group of neurological conditions characterized by abnormal voluntary or involuntary movements also recurring in Cerebral Palsy (CP), a permanent non progressive neurodevelopmental condition involving movement and posture alterations. Although the etiology is often unknown, this spectrum of conditions has a strong genetic component. The aim of this study was to design a customized targeted gene panel to investigate the genetic bases of CP and MD with childhood onset. The panel includes 59 genes considered disease-causing or related with primitive MD/CP conditions. In 9 of 18 subjects tested so far, we selected 14 new or low-frequency single nucleotide variants (SNVs) in 10 of the 59 selected genes. Five variants in genes associated with pediatric-onset MD (*ANO3*, *AP4S1*, *AP4E1* and *VPS13D*), two (one missense and one intronic) in *GAD1* a gene considered to be associated to CP, and seven missense variants in candidate CP genes (*ITPR1, NAA35, MCPH1, PACRG, SCN8A, SPTBN2* and *TENM1*).A homozygous truncating mutation, already described in literature, was found in *AP4S1*. A few potentially disease-related variants were detected in genes associated with recessive phenotypes: an apparently homozygous missense variant in *MCPH1* suspected to mask a heterozygous gene deletion is currently under characterization. A *VPS13D* and a *GAD1* likely pathogenic variants were detected as compound heterozygous in association respectively with a samesense and an intronic VUS both predicted as possibly altering splicing mechanisms. Although preliminary these results may contribute to the recently highlighted evidence of a strong genetic base for these rare disorders.

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P09.081D
The Genetic Basis of paediatric movement disorders: experience from the SYNaPS Study

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Introduction: Pediatric movement disorders which are usually part of complex neurodevelopmental disorders comprise a wide group of neurological diseases with highly variable, often complex clinical presentation. Although causative mutations in >200 genes have been associated with various movement disorders, many patients remain without a precise genetic diagnosis. The SYNaPS Study, which is IRB/ethics approved and aimed at analysing unexplained ultra-rare neurological conditions, aim to identify underlying genetic causes in patients with pediatric movement disorders by high-throughput genetic investigations.

Materials and Methods: As part of SYNaPS study a large cohort of well-phenotyped families recruited from multiple paediatric neurology clinics around the world with a diverse ethnic background affected by different childhood

and early-onset movement disorders were genetically and clinically investigated. exome sequencing was performed for probands of around 500 families with any forms of movement disorders. Some of the unsolved individuals were subjected to a combination of SNP-Array genotyping/ homozygosity mapping, whole genome sequencing and long-read sequencing.

Results: Overall, in this study we resolved around 50% of the patients with movement disorders. We also uncovered novel disease-causing genes in various families. Re-annotating/re-analysing the exome data along with more extensive data sharing and also employing homozygosity mapping in some of the families increased the rate of diagnosis.

Discussion: We made a molecular diagnosis for around half of the families and characterised multiple new genes and ultra-rare movement disorders. A genetic diagnosis provided either disease-specific treatment or effected management for some patients with a genetic diagnosis, highlighting the importance of early and specific diagnosis.

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P09.082A
Evaluating motor functions and biomarkers for Fukuyama muscular dystrophy

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Fukuyama muscular dystrophy (FCMD) is an autosomal recessive, severe childhood muscular dystrophy with brain anomaly. FCMD is mainly caused by an ancestral insertion of 3-kb retrotransposal element into the 3' untranslated region of the causative gene, *fukutin*. Recently, we testified that pathogenic exon trapping by the transposon cause splicing abnormality in FCMD. We have testified that antisense oligonucleotides targeting this splicing could be a possible therapy in the future. However, there has been no biomarkers or few report on natural history for assessing the disease level of FCMD. To find specific serum biomarkers and to comprehend natural history of FCMD patients, we collected serum and clinical data from patients. We tested on serum biomarkers by measuring muscle specific microRNAs. Clinical data contains motor function score, muscle elastography (shear wave elastography (SWE)), and whole body muscle computed tomography with ultralow level irradiation. As a result, miR206 was significantly overexpressed in FCMD patients compared to normal controls. Correlation coefficient of miR206 with serum CK level, serum creatinine level, and motor function scores were also high. MiR 206 was especially high in FCMD patients with high muscle contents, suggesting remaining sparing capacity of muscle regeneration. SWE showed significantly high elasticity in biceps brachii and brachial muscle but not high in lower extremities in FCMD patients, compared to normal control, because of high content of fat infiltration due to disuse of lower extremities. In conclusion, serum miR206 and SWE is useful for monitoring muscle wasting progression and motor function level of FCMD.

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P09.083B
New genes involved in diseases with neurodegeneration with brain iron accumulation (NBIA)

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Introduction: Neurodegeneration with brain iron accumulation (NBIA) is a heterogeneous group of inherited neurologic disorders characterized by progressive movement disorders and abnormal accumulation of iron in the basal ganglia. There are 12 genes that resolve 80% of cases. Our clinical series comprises 134 probands who suffered from NBIA and other movement disorders. After analyzing the NBIA genes, we have achieved the genetic diagnosis in 54 cases. The aim of the present study is to establish the molecular bases in patients without mutation in known NBIA genes.

Materials and Methods: Thirty-eight patients have been studied by a customized gene panel based on SureSelect Technology (Agilent), which comprises 498 genes involved in NBIA, ataxia, chorea, dystonia, parkinson, spastic ataxia, and spastic paraplegia.

Results: We have achieved a definite diagnosis in 19 probands. Four of them presented with a neuroimaging phenotype suggestive of NBIA and carried novel mutations in *GLB1, FUCA1* and *TPP1*, and in one patient born to consanguineous parents, homozygote mutations were identified in two genes, *FBOX7* and *DLD*.

Conclusions: We have solved 50% of cases. Except for *FUCA1*, these are the first cases described of NBIA with mutations in *GLB1, TPP1*, and *DLD/FBXO7*. According new cases are reported, the clinical and genetic spectrum associated with NBIA are expanding. *Funding: Fundació La Marató de la TV3, PROMETEO/2018/135, ISCIII-PI15/00187 and ISCIII-P18/00147, cofund with FEDER funds*.

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P09.084C
Strong interest of exome sequencing in progressive neurological diseases

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Introduction: Neurogenetics represents a vast, complex, ever changing discipline whose diagnosis currently remains challenging, since clinical and/or imaging features frequently appear very unspecific, especially early in the evolution (cerebellar ataxia, tremor, dystonia···). In molecular diagnosis, current strategies usually include sequential investigations that may lead to long, tedious, expensive and disappointing patients care. Exome sequencing (ES) appears a promising approach for neurogenetics, apart from when nucleotide motif expansion disorders can be suspected.

Materials and Methods: We recruited 48 individuals without cognitive development impairment, referred to our center for suspected neurogenetic disease: 20 cerebellar ataxia (42%), 12 neuromuscular diseases (25%), 8 spastic paraplegia (17%), 2 abnormal movements (4%) and 6 others (12%) for whom the phenotype could not be labelled under a usual neurological syndrome. ES was interpreted in a solo-based strategy (94%) or in trio with parental pool (6%).

Results: ES identified a causal diagnosis in 4/8 individuals with spastic paraplegia (50%), 3/6 "other" (50%), 1/2 with abnormal movements (50%), 5/12 with neuromuscular diseases (42%), 4/11 with isolated cerebellar ataxia (37%) and 2/9 with spinocerebellar ataxia (22%). Overall diagnostic yield was of 40 %.

Conclusions: With such overall diagnostic yield, this study reinforces the diagnostic interest of ES in neurogenetics, in all its fields, as this diagnostic yield ranges from 22% in spinocerebellar ataxia (which is higher than current yield of gene panels) to 50% in spastic paraplegia. It also includes situations in which clinical displays may be complex and hard to systematize. First-tier implementation would significantly improve diagnostic yield in neurogenetics.

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P09.085D
Elevated impact of chromatin regulating genes in the genetic diagnosis of neurodevelopmental disabilities

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Introduction: Exome trio analysis is an effective strategy to identify potential causal variants in rare genetic disorders with clinical heterogeneity. In this study, we focused on the 404 probands with neurodevelopmental disabilities (NDD) with causal variants on chromatin-regulating genes to determine their frequency and their genotypic/phenotypic characteristics by trio analysis.

Patients and Methods: A cohort of 259 males and 145 females, with a median of 7 years old, was studied by trio whole exome sequencing. Libraries were generated with Ion AmpliSeq™ or SureSelect^{XT} and sequenced with Ion

Proton™/S5™XL or NovaSeq 6000. The data were processed using an in-house pipeline.

Results: A genetic diagnosis was established in 129 of the 404 studied probands, leading to a diagnostic yield of 32%. Among the positive cases, 82 and 47 variants, were considered as casual or probably casual, respectively. Of that diagnosed patients cohort, 26 probands (20%) had variants in chromatin-regulating genes (ARID1B, ATRX, EHMT1, SATB2, SMARCA2, and KMT family). Of the 26 variants identified, 58% were loss of function de novo variants associated with an autosomal dominant inheritance. Clinically, these patients shared NDDs phenotypic characteristics (psychomotor delay and/or autism spectrum disorder) with short stature, dysmorphic features, epilepsy and/or digital malformations.

Conclusions: The haploinsufficiency associated with loss of function variants in the genes implicated in the regulation of chromatin played a crucial role in neurodevelopment disorders. These deleterious variants seemed to be associated with a common phenotype. A better delineation of these clinical manifestations could help to better recognize them in the clinical setting.

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P09.086A
Novel candidate genes in autosomal recessive neurodevelopmental disorders: A three year cohort study

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In a three-year cohort study, 313 Families with autosomal recessive pattern compatible with genetic disorders such as seizures, hypotonia, neurodegenerative disorder, progressive brain atrophy, developmental regression have been conducted. The progression in diagnosis of neurodevelopmental disorders has undergone considerable in the past decade. In this cohort study we aim to explain our broad research on identification of six novel candidate genes (PPP1R21, ADPRHL2, APC2, SLC10A7, APC2, DEAF1) on the patients with autosomal recessive neurodevelopmental disorders. A complete clinical and paraclinical examination has been done by expert specialists and clinical geneticist. Exome Sequencing was performed and followed by bioinformatic analysis. Parents and healthy offspring were assessed for the candidate gene variants. We delineated a novel neurodevelopmental disorders caused by biallelic PPP1R21 loss of function variants, and identified four previously unreported homozygous truncating PPP1R21 alleles. Pathogenic variants have been identified in a number of patients presenting broad clinical phenotypes with autosomal recessive inheritance. By using linkage analysis and exome or genome sequencing, recessive inactivating mutations in ADPRHL2 in six families have been recognized. It has been demonstrated that SLC10A7 mutations reduce SLC10A7 protein expression by in vitro studies. We also described the participation of the APC2 and DEAF1 Genes as potential functional candidates in neurodevelopmental diseases based on computational prediction by using several cellular tools. The current focus of our research is on neurodevelopmental disorders, especially autosomal recessive. To further, workup with next generation technologies, using several cellular tools is essential for precise phenotype definition and to understand the underlying disease mechanisms

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P09.087B
Neurofibromatosis type 1 mutational spectrum in Macedonian patients: A report of seven novel pathogenic variants

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Introduction: Pathogenic variants in *NF1* gene are associated with autosomal dominant Neurofibromatosis type 1 (NF1) which is a multi-systemic, neurocutaneous disorder with predisposition to benign or malignant lesions of the skin, nervous system and bone. Here we present the first genetic study of NF1 in R. Macedonia.

Material and Methods: Since 2014, a total of 33 patients suspected for NF1 were referred to our laboratory. The methodology included cDNA sequencing, next-generation sequencing using Illumina's TruSight Cancer panel, direct DNA sequencing and multiplex ligation probe amplification.

Results: A total of 21 different pathogenic variants were detected in 22 unrelated patients. The variants comprised nine nonsense, six frameshift, two splicing, two missense

mutations, one multi-exon deletion (exons 9-51) and one 1.4 Mb deletion including the entire *NF1* gene. All variants were unique, detected in one patient/family with the exception of 1.4 Mb deletion which was detected in two unrelated patients. Genetic testing of family members was available for 20 patients; the *NF1* variation was inherited from an affected parent in 11 (55%), while in nine patients (45%) it has arisen as a *de novo* event. Seven mutations were novel: one nonsense (c.5844C>G, p.Tyr1948Ter) and six frameshift mutations that cause premature termination of the protein (c.208delA; c.1104_1107delTCAG; c.1480_1481delTT; c.2495_2496dupAC; c.4517delC; c.6971delA). Five of the novel mutations were inherited from an affected parent, while c.2495_2496dupAC and c.6971delA have occurred *de novo*.

Conclusion: Our study presents the *NF1* mutational spectrum in R. Macedonia and expands the global spectrum of *NF1* pathogenic variants.

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P09.088C

A novel *IRF2BPL* truncating variant is responsible for infantile neuronal ceroid lipofuscinosis-like phenotype

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Introduction: Neuronal ceroid lipofuscinoses (NCLs) are neurodegenerative lysosomal storage disorders characterized by the progressive loss of both motor and cognitive skills, myoclonic epilepsy and the presence of lysosomal deposits with characteristic ultrastructural features, due to mutations in thirteen distinct genes. Here, we report a child presenting a neurological disorder characterized by motor and cognitive regression, spastic-dystonic tetraparesis, sporadic myoclonus, typical EEG abnormalities and lysosome deposits of granular osmiophilic material with tubular and fingerprint-like structures. A diagnosis of NCL was suspected, but genetic analysis of known NCL genes yielded negative results.

Materials and Methods: Whole exome sequencing was performed on genomic DNA of the proband and both parents, upon written informed consent. Variants were aligned, filtered and prioritized to identify candidate variants. Validation and segregation analysis were performed with Sanger sequencing.

Results: The proband was found to carry a novel heterozygous truncating variant in the *IRF2BPL* (Interferon regulatory factor 2 binding protein-like) gene, arisen *de novo*. Very recently, *de novo* heterozygous variants in this gene have been reported in 18 patients showing neurodegenerative phenotypes variably defined as neurodevelopmental disorders with regression, abnormal movements, loss of speech and seizures or developmental epileptic encephalopathies.

Conclusions: This case expands the phenotypic spectrum of *IRF2BPL* to include NCL-like phenotypes.

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P09.089D

Genetic analysis of early-onset schizophrenia

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Introduction: Early onset schizophrenia (EOS) is rare complex psychiatric disorder with polygenic inheritance. Preliminary data suggest that EOS has higher genetic liability to the disease. Therefore, we preformed genetic testing using both molecular karyotyping (arrayCGH) and next generation sequencing (NGS) for determination of copy number variations (CNVs) and single nucleotide variants (SNVs),

affecting the possible schizophrenia-associated genes in a small group of Slovenian children diagnosed with EOS.

Material and Methods: Our study included 49 patients. In all patients molecular karyotyping was performed, following the medical exome sequencing (Illumina TruSight One capture). Considering the polygenicity of the disorder, we also analysed enrichment of the list of genes harbouring possibly deleterious variants in Gene Ontology terms (GO) by using GeneMania version 3.4.1 Cytoscape plugin.

Results: In 12% (6/49) of children a clinically significant CNV was detected, that could be associated with the disorder. In 14% (6/43) of patients pathogenic SNV were detected, although no clear genotype-phenotype correlations could be made. Therefore, using the GO terms, we assembled a list of genes harbouring probably deleterious variants.

Conclusion: The aim of this study was to assemble a list of genes harbouring probably pathogenic variants in Slovenian patients with EOS by performing the molecular karyotyping and NGS method. A 12% of patients are carriers of clinically significant CNV. NGS analysis and enrichment analysis showed that gene ontology terms related to schizophrenia are enriched in genes, selected by our filtering. The over-represented pathways could be associated with pathology of schizophrenia in Slovenian population.

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P09.090A

Diagnostic yield of ngs-based epilepsy and neuromuscular disease panel in russia population

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Single nucleotide variants (SNVs) are a common cause of congenital anomalies, developmental delay, epilepsy, neuromuscular disorders, cardiovascular and other disorders. In 2014-2019 we are performed 6846 NGS-based tests for patients with suspected monogenic disorders using Illumina NextSeq 500 and in-house analysis software. 3110 samples were sequenced using Illumina TruSightOne enrichment ("clinical exome"); 594 samples by whole-exome sequencing; 3142 samples using our custom 2000-genes platform, focused on epilepsy and neuromuscular disorders. A definitive molecular diagnosis could be estimated in 2238 cases (32,69%), 1043 (15,24%) having a possible diagnosis with a need for additional testing. We compare a diagnostic yield our epilepsy panel (Table 1) and neuromuscular diseases panel (Table 2) with results of other published studies.

Table 1

	Genomed	Kodera et al., 2013	Trump et al., 2016	Møller et al., 2016	Butler et al., 2017
Definitive diagnosis	27,7%	23%	18,58%	22,67%	18,29%
Possible diagnosis	15,48%				
No diagnosis	56,82%				
Gene count	1081	35	46	46	110
Number of patients	1693	53	323	216	339

Table 2

	Genomed	Evilä et al., 2016	Chae et al., 2015	Dai et al., 2015
Definitive diagnosis	44,67%	15%	48,8%	65%
Possible diagnosis	12,12%			
No diagnosis	43,21%			
Gene count	836	180	579	399
Number of patients	553	61	43	55

Mutation frequency analysis showed that the top 20 genes account for 57,51% and 46,72% definitive diagnosis in epilepsy and neuromuscular disease panel respectively. Nevertheless, the use of large panels is justified due to higher diagnostic yield and cost-effectiveness.

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P09.091B

Identification of potential new genes involved in autosomal recessive form of Parkinson's disease

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Parkinson disease (PD) affects 1% of the population above 65 years. It is characterized by the triad of symptoms: tremor, rigidity, and bradykinesia. To date the identified genes associated with early-onset (EO, >40 years) autosomal recessive (AR) PD only explain 45%, other genes remain to be discovered. The aim of the work is to identify new genes involved in AR EO PD, using consanguineous PD families and applying genotyping on DNA microarrays and NGS technologies.

Using a series of 99 families with confirmed consanguinity, we looked for homozygous loss of function or missense mutations predicted deleterious in region of loss of homozygosity. Then we first focused on variant shared by at least two families. We identified mutations in *PSMF1* an interactor of *FBXO7*. In one family, only this variant remains, moreover both mutations code for amino acid highly conserved upon evolution.

Most of the candidate's genes are private genes highlighting genetic heterogeneity of PD. Therefore, in a second time we hypothesized that some candidate's genes can be involved in a common pathway. Using ClusterProfiler we performed GO term enrichment analyses, then we were able to grouped together some genes and were able to see an statistical enrichment in autophagy pathway.

We identified a strong candidate gene for AR-PD: *PSMF1*. Further functional data are needed to strengthen the role of this gene in PD, possibly affecting the proteasome activity and α-synucleine aggregation. We will also continue to investigate pathway analyses in order to identify candidates for PD in our families.

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P09.093D

PEHO syndrome caused by compound heterozygote variants in *ZNHIT3* gene

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PEHO syndrome (MIM 260565) is characterized by progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy, first described in 14 Finnish patients. A homozygous missense substitution c.92C>T (p.Ser31Leu) in *ZNHIT3* was identified as the primary cause of PEHO syndrome in Finland.

Case report: The index patient was hospitalised at 1m due to muscular hypotonia and lethargy. Brain MRI showed mildly dilated lateral ventricles, a porencephalic cyst on the left, hypoplastic occipital lobes and absence of myelin in the cerebellar white matter. At 10m, generalized seizures started (myoclonus and infantile spasms). EEG showed hypsarrhythmia. The child was blind at 17m; she had pale and small disks, and temporal disk pallor. At 8y, she has severe microcephaly (-4SD), no active movements, severe spasticity, and scoliosis. Her face is edematous with an opened mouth appearance. Brain MRI showed severe cerebral, cerebellar and brainstem atrophy, thin and short corpus callosum and almost absent periventricular white matter. To identify the underlying etiology of this phenotype, trio exome was performed and negative, after which trio genome sequencing was performed.

Result: Two high quality heterozygous missense variants in *ZNHIT3* gene were identified: the c.92C>T p.(Ser31Leu) variant (NM_004773.3), which was previously described in Finnish patients and the novel variant c.41G>T p.(Cys14-Phe). There are eight heterozygotes and no homozygotes for the latter variant in gnomAD database and multiple *in-silico* pathogenicity predicting algorithms indicated a damaging effect.

Conclusion: We reported the first patient outside Finland with confirmed *ZNHIT3* variants causing PEHO syndrome. Funding: Estonian Research Council grants PUT355, PRG471, and PUTJD827.

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P09.094A

Exome-based bottom-up approach of molecular diagnostics in a clinically ambiguous family with a polystigmatized patient revealed ultra-rare *NEDD4L* associated periventricular nodular heterotopia

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Introduction: Periventricular nodular heterotopia-7 (PVNH7) is an ultra-rare neurodegenerative disorder affecting proper neuronal migration during neurogenesis. Only seven patients carrying pathogenic variants in the *NEDD4L* gene, associated with PVNH7, were reported so far. We report a polystigmatized 2-year-old boy having significant symptomatologic overlap with PVNH7, however, showing also symptoms falling outside PVNH7 symptomatology. His brother presents with some of the proband´s symptoms, specifically those falling outside PVNH7 symptomatology.

Materials and Methods: The boy, his brother and their parents were subjected to whole-exome sequencing. Variant prioritization was performed using an inheritance-pattern and phenotype-driven top-down approach, or by a bottom-up approach, beginning with the identified known pathogenic variants in the proband's data.

Results: Because of uncertainties in symptomatology and inheritance pattern, the top-down approach was hard to apply. The bottom-up approach, however, identified a known pathogenic variant, NM_001144967.2(NEDD4L): c.2677G>A:p.Glu893Lys, in the proband's genome, absenting in any other analyzed family member. This *de novo* variant explained symptoms overlapping those reported for PVNH7, such as delayed psychomotor development and intellectual disability, absenting speech and walking, hypotonia, strabismus, cleft palate, microretrognathia, and 2-3 toe syndactyly. Symptoms shared with the brother, on the other hand, were not explained by this variant. These are most probably not extended phenotypes of PVNH7, rather an independent clinical entity.

Conclusion: Our case highlights: 1) the usefulness of a bottom-up approach of causative variant identification during differential diagnostics of patients/families having unclear/overlapping phenotypes; 2) the importance of reporting yet undescribed symptoms of known disorders, found in single patients/families, with special care.

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P09.095B
Pitt-Hopkins syndrome caused by a pathogenic splicing variant

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Introduction: Pitt-Hopkins syndrome is a rare inherited neurological condition, associated with heterozygous pathogenic variant in the *TCF4* gene or with deletion of the 18q21.2 chromosome region, in which the *TCF4* gene is located. In the present study we report a novel clinical case of a 7-year-old patient with global development delay and multiply minor dysmorphic features.

Materials and Methods: Whole exome sequencing was performed on Ion S5 next-generation sequencing system. The identified variant was confirmed by Sanger sequencing. RT-PCR analysis was performed using total RNA isolated from patient fibroblasts. HEK293 cells were transfected with minigene plasmid vector containing the variant of interest. Splicing alterations were validated using RT-PCR with further Sanger sequencing.

Results: Whole exome sequencing revealed a previously undescribed heterozygous variant in the intron 11 of the *TCF4* gene - NM_001083962: c.922+5G>C. Segregation analysis showed that the variant is *de novo*. Functional analysis using two independent approaches: RT-PCR analysis of total RNA extracted from patient fibroblast and a splicing minigene assay, showed that c.922+5G>C variant disrupt the donor splicing site of the intron 11 leading to a complete skipping of exon 11, resulting in a frame shift and premature stop codon formation p.(Ser264GlnFsTer83).

Conclusion: Therefore, after performing functional analysis we classify the c.922+5G>C variant as pathogenic and disease causing in our patient.

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P09.096C
Expanding the phenotypic spectrum associated to PMPCA mutations

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Introduction: Biallelic missense mutations in the *PMPCA* gene, encoding mitochondrial processing peptidase alpha-subunit, were initially identified in 19 individuals from five families with early onset, non-progressive cerebellar ataxia, developmental delay and intellectual disability. Brain MRI showed non-progressive cerebellar atrophy; muscle biopsy was normal, although mitochondrial complexes activity was mildly decreased in some cases. Subsequently, two cousins were reported with a more severe phenotype of progressive mitochondrial encephalopathy, including profound developmental delay, ptosis, ophthalmoplegia, weakness, respiratory insufficiency, blindness and lactic acidemia. Brain MRI showed progressive cerebral-cerebellar atrophy, with a lactate peak at spectroscopy. Muscle biopsy demonstrated enlarged, structurally abnormal mitochondria. Finally, a 7-year-old boy was recently reported with psychomotor delay, spastic-ataxic gait and regression. He had elevated blood lactate and abnormal muscle biopsy with mitochondrial damage. Besides cerebellar atrophy, brain MRI showed bilateral symmetric hyperintensity in the striatum (Leigh-like features).

Case Report: We report a 12-year-old girl carrying two novel, likely pathogenic PMPCA missense variants, who presented a complex, non-progressive early-onset phenotype of spastic ataxia with superimposed generalized hyperkinetic movements, intellectual disability, microcephaly and epilepsy. Several brain MRIs showed non-progressive cerebellar atrophy and bilateral symmetric T2-hyperintensity and hypotrophy of both caudate and putamen, resembling striatal necrosis. Blood lactate and MRI spectroscopy were normal.

Conclusions: This case further expands the clinical spectrum associated with *PMPCA* mutations to include a complex encephalopathy without obvious mitochondrial involvement. The association of non-progressive cerebellar atrophy with bilateral striatal hyperintensity may represent a "red flag" for this genetic condition.

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Novel association of biallelic *ATOH1* variants with pontocerebellar hypoplasia, developmental delay and hearing loss

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The pontocerebellar hypoplasias (PCH) represent a heterogeneous group of conditions characterized by structural abnormalities of central nervous system, typically involving cerebellum and ventral pons. In the present study, we propose a novel association between biallelic variants in ATOH1 and PCH with severe neurodevelopmental disorder. We report a family with two children affected with pontocerebellar dysplasia, severe motor and speech delay and sensorineural hearing loss. Using exome sequencing, we identified a homozygous rare missense variant in ATOH1 in both affected probands. ATOH1 encodes atonal bHLH transcription factor 1, a core neurogenic transcription factor that regulates cell differentiation in cerebellum, drives development of inner ear hair cells and is essential in development of neurosensory systems. Disruption of Atoh1 in mouse models was previously shown to result in severe cerebellar hypoplasia and hearing loss. The identified variant (NM_005172.1:c.481C>G, p.Arg161Gly) substitutes a highly conserved residue in the DNA binding domain of Atoh1. It is absent from control populations of the gnomAD project and our in-house database of 3000 exomes. We performed molecular modelling, which predicted that this change can affect DNA binding affinity of Atoh1 through distortion of its contacts to DNA and through remodelling of the helical part of HLH domain. In conclusion, we report a novel potential genetic cause of developmental brain anomalies, due to mutations in ATOH1. Although the evidence is currently too limited to conclusively establish its causality, this report may present basis for further studies on the role of ATOH1 in human disease.

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P09.098A

The effect of APOE in Alzheimer's disease is age dependent; demonstrated in the UK Biobank data

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Alzheimer's disease (AD) is a devastating neurodegenerative condition with significant genetic heritability. The apolipoprotein E (*APOE*) gene is the strongest genetic risk factor for AD. The risk of *APOE* in AD varies with age. This age-related genetic heterogeneity has been shown in the Alzheimer's Disease Genetics Consortium data; there is little genetic correlation between two age groups (60-79 years vs. 80+ years) and the heritability explained by SNPs on chromosome-19 was larger at younger ages.

We aim to further demonstrate that *APOE* impacts the risk of AD in younger subjects, whereas older subjects are influenced by the polygenic effect of variants outside the *APOE* region.

The UK Biobank data is a large (N=443,019) prospective cohort of individuals containing genetic data for 7,654,308 imputed SNPs. Individuals in Biobank are relatively young, with only 92 AD cases. Polygenic risk score (PRS) is a method to combine the effect of genetic variants. We computed the PRS for individuals in Biobank, using summary statistics from the largest AD GWAS to weight the score.

AD PRS shows an association with AD, but this effect is removed when the PRS excludes chromosome-19. When considering family proxies of AD; sibling proxies show the same pattern, however, parental proxies remain associated with PRS even after the removal of chromosome-19. This is similarly shown in biological pathways; beta-amyloid regulation, protein-lipid complex and APP regulation. This is also demonstrated using age-stratified analyses which display a stronger association with AD and higher mean PRS in AD cases in the older cohort.

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P09.099B

DNA methylation as link between life adversities and emergence of psychopathology

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DNA methylation plays a role in the regulation of gene expression in response to environmental factors. A previous longitudinal study of our group found 66 genes differentially expressed in the blood of youths who presented an increase of dimensional psychopathology after a 3-year follow-up. These genes were exclusively found in the group with the emergence of psychopathology and were not differentially expressed in the longitudinal control groups. Here, we aimed to identify whether the gene expression of these 65 genes were influenced by changes in DNA methylation in the blood of the same individuals. Moreover, for those markers associated with gene expression, we verified whether DNA methylation was influenced by adversities experienced by the youths during the 3-year follow-up. Then, we compared gene expression and DNA methylation data in peripheral blood samples (n=44) obtained from 22 youths before and after developing severe psychopathology. Life adversities variables were generated by using a latent modelling approach with a bifactor structure. We identified 11 differentially expressed genes regulated by DNA methylation. Among these 11 genes, the methylation of *LMF2* and *ETS1* was influenced by factors describing adversities related to interpersonal, context change, school, health/loss and unpredictable events. Also, these influences persisted when modelling a general adversity factor. *LMF2* methylation has been associated with Alzheimer's disease and *ETS1* is a transcriptional factor involved in downstream biological pathways. These results provide further evidence that life adversities and the emergence of psychopathology are functionally linked by changes in DNA methylation and, consequently, in gene expression.

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P09.100C

No individual prediction of clinical outcome of ultra-high-risk individuals by the polygenic risk scores

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Introduction: The identification of clinical ultra-high-risk state for psychosis (UHR) is now allowed in clinical practice by assessing attenuated symptoms. Prediction of the emergence of psychosis and dimensional clinical outcomes is important for early intervention. The results of recent large-scale genome-wide association studies (GWAS) expanded our knowledge of the genetic factors and suggested a polygenic model of inheritance of psychiatric disorders. We aimed to test if the individual polygenic risk scores (PRS) could predict for conversion to psychosis and dimensional outcomes and if the converters to psychosis have polygenic risk for psychiatric disorders.

Materials and Methods: In the French cohort ICAAR, we collected longitudinal data of 104 individuals with at-risk mental state enrolled in a 1-year follow-up study. Clinical assessments included CAARMS, PANSS, MADRS, YMRS, SOFAS and CGI. The conversion to psychosis was characterized using the CAARMS-defined psychosis onset threshold. We genotyped more than 500,000 common variants and computed four different PRS based on the data of four GWAS from the Psychiatric Genomics Consortium.

Results: The PRS from currently available GWAS were poor predictors of the conversion to psychosis, and there was no significant difference between converters and non-converters. The PRS were not correlated with any phenotypic scale at the baseline measurement (M0) and at the last measurement (after 6 months or 12 months).

Conclusion: The PRS cannot be used in clinical practice to predict the individual risk of conversion to psychosis. Further studies are needed to examine the correlation between genetic variance and clinical outcomes in psychiatric disorders.

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P09.101D

Mineral absorption is an enriched pathway in the brain regions of restless legs syndrome patients with lower *MEIS1* expression

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Restless legs syndrome (RLS) is a common complex disorder for which different genes and environmental elements are considered risk factors. Such a mixed nature makes it challenging to characterize the defective pathways associated with the condition. Here we conducted a genome-wide transcriptome (RNA-Seq) study and observed how the gene reported to present the most significant association with RLS, *MEIS1*, acts as a regulator over the expression of additional genes. Interestingly these additional genes appear to entail some of the pathogenic pathways known to be linked to RLS. Our data revealed *HMOX1*, *TFR2* and *VDR*, three genes encoding products with RLS relevant functions, to be regulated by MEIS1 in human cells where the expression of *MEIS1* was either increased or prevented. These manipulations of *MEIS1* in cells revealed mineral absorption to be an enriched pathway in regard to MEIS1 regulatory role as a transcription factor. The same enrichment is observed in postmortem brain regions (thalamus and pons) obtained from a subset of RLS patients presenting changes in MEIS1 expression. The expression of genes encoding metallothioneins (MTs) was observed to be affected across the different RNA-Seq datasets. MTs are highly relevant to RLS as they bind intracellular metals and interact with ferritins which manage iron levels. Overall, our study suggests that in a subset of patients, the contribution of *MEIS1* to RLS is associated to its transcriptional regulation of other genes more directly involved in cellular pathways relevant to RLS.

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P09.102A

Alterations in GABA A1 Receptor in Rett syndrome: the necessity of early GABAergic modulation as a therapeutic strategy

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Introduction: Rett syndrome (RTT; OMIM#312750) is a severe neurological disorder which mainly affects young females and is usually caused by mutations in the gene *MECP2*. However, not much is known about which pathways can be disrupted in RTT downstream MeCP2 dysfunction. Nevertheless, recent studies demonstrate the possible implication of a disturbance in inhibitory circuits, especially of the GABAergic inhibitory neurons, in RTT pathophysiology.

Methods: This work analyses changes in expression of the most common subunits of GABA(A) ionotropic receptors in different models of RTT (*in vitro* cell culture and *in vivo* mouse model) by shRNA knock-down, qRT-PCR, western blot and immunocytochemistry, as well as in post-mortem brain samples of RTT patients by RNAseq, with the aim of finding a suitable therapeutic target for treating RTT.

Results: We observed a direct relationship between MeCP2 and GABA (A) receptor subunit a1 (Gabra1) expression in cell lines and primary cultured neurons. Protein analyses of female $Mecp2^{+/-}$ and control mice at different developmental stages confirmed our previous findings and pointed towards the importance of the developmental status in this relationship, highlighting synaptic protein expression changes in presymptomatic stages of RTT. RNAseq results in brain samples of RTT patients confirmed the importance of the time frame when studying RTT unbalanced neurotransmission.

Conclusions: Our results strongly support Gabra1 as a novel therapeutic target for treating RTT. Protein expression changes in presymptomatic stages of RTT highlight the importance of early therapeutic strategies targeting neurotransmission. Grants: Mi Princesa Rett and FIS (PI15/01159, PI16/00851 and PI15/01082 ISCIII and FEDER).

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P09.103B

Absence of Heterozygosity Flags *BRAT1* and Leads to the Diagnosis of Neonatal Lethal Rigidity and Multifocal Seizure Syndrome

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Neonatal Lethal Rigidity and Multifocal Seizure Syndrome (RMFSL) is a rare autosomal recessive condition characterized by intractable seizures, hypertonia, autonomic instability and early death. Mutations in *BRAT1* (Breast Cancer 1-associated ataxia telangiectasia mutated activation-1) have been associated with severe RMFSL as well as related phenotypes of varying clinical severity. We report on a female baby who presented with mild dysmorphic features, striking hypertonicity, seizures with a burst suppression pattern on EEG and apneic spells shortly after birth who died at 2 months of age. Chromosomal microarray analysis using a combined non-polymorphic /SNP probe based platform (Affymetrix CytoScan HD) did not identify any copy number variations but several stretches of absence of heterozygosity (AOH) were identified including a region on chromosome 7p22.3p22.1. As *BRAT1* is contained within this region and the clinical phenotype was suggestive of RMFSL, *BRAT1* sequencing was performed. A homozygous, previously unreported pathogenic mutation (c.1013dupC) was identified, confirming the diagnosis of RFMSL. To our knowledge, this is the seventh report of RFMSL due to a homozygous *BRAT1* mutation in the literature, and the first one reported in a family of French Acadian background from Atlantic Canada. This case demonstrates the utility of SNP-based microarrays which can detect regions of AOH and help narrow the differential diagnosis and approach to subsequent diagnostic investigations.

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Changes in expression levels of some microRNAs in the rat frontal lobe at different times of healing period after experimental subarachnoid hemorrhage

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Introduction: The expression of certain miRNAs, such as miR-132, miR-134 and miR-138, which are thought to be associated with synaptic plasticity and neurological functions, can affect the density and volume of dendritic spines. In this study, it was aimed to observe how the levels of different miRNAs in the frontal lobes of experimental subarachnoid hemorrhage (SAH) induced rats were changed in different time intervals

Materials and Methods: Adult female Sprague-Dawley rats (n=21) are divided into 3 groups: control-group (n=7), 7th day post-SAH-group (n=7) and 14th day post-SAH-group (n=7). Experimental-SAH was induced in SAH-groups. The levels of miR-132, miR-134 and miR-138 in the frontal lobes were determined by qPCR and statistical differences were calculated using one-way ANOVA test.

Results: A statistically significant increase in expression levels of miR-132,miR-134 and miR-138 was observed on day7 post-SAH ($p<0,001$). On the 14th day, it was determined that the level of miRNAs which were increased earlier, decreased to the control values ($p>0.05$).

Conclusions: Expressions of microRNAs are elevated during 1-7 days post-SAH, and decreased again in the interval of 7-14 days. These results show that miR-132, miR-134 and miR-138 levels were affected during the first week of recovery post-SAH. This increase in microRNA levels, which have different effects on synaptic connections, in the short-term after SAH may play a role in the inaccurate arrangement of synaptic structures and in the emergence of neurological dysfunctions. Further studies needed to support our data.

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P09.105D
Screening for spinocerebellar ataxia type 36 (SCA36) in the Greek population

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Introduction: Spinocerebellar ataxia type 36 (SCA36) is an autosomal dominant (AD) disorder, characterized by late-onset cerebellar ataxia (5th and 6th decade of life), dysarthria, sensorineural hearing loss and lower motor neuron involvement, particularly tongue atrophy. A GGCCTG hexanucleotide repeat expansion in intron1 of the *NOP56* gene causing RNA gain of function is responsible for the disease. Normal size alleles vary from 3 to 14 repeats, pathogenic alleles have more than 650 repeats, and intermediate alleles are of uncertain significance.

Materials and Methods: Our cohort consisted of 98 selected index patients. 92 patients came from an ataxia cohort (n=600), negative for the most common SCAs (SCA1, SCA2, SCA3, SCA6, SCA7), with pedigree and phenotype consistent with SCA36 and 6 came from a suspected Kennedy's disease cohort (n=200), negative for the CAG trinucleotide repeat in *AR* and pedigree consistent with SCA36. The number of GGCCTG hexanucleotide repeats was determined with conventional PCR for the smaller alleles and RP-PCR for the larger alleles. Fragment length analysis was performed and positive controls were used in every run.

Results: No pathologic repeat expansions in *NOP56* were detected in our cohort.

Conclusion: Supplementary to our previous study of AD spinocerebellar ataxias in the Greek population, we screened a less common gene causing inherited ataxia. In line with SCA3, SCA36 seems to be a very rare finding in the Greek population.

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P09.106A
Rare genetic variants in *SHANK1* and *SHANK2* genes found in Bulgarian patients with schizophrenia and bipolar disorder

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Genes associated in postsynaptic plasticity have been shown to contribute to schizophrenia (SCZ) and bipolar affective disorder (BAD), so is reasonable to hypothesize that the etiology of psychiatric diseases is linked to synapse structure, transmission and postsynaptic density. Shanks are adapter proteins in the postsynaptic density that interconnect receptors of the postsynaptic membrane.The members

of *SHANK* gene family are candidate genes for psychiatric disorders.

A total of 300 individuals with BAD, 151 with SCZ, 15SAD diagnosed based on DSMIV, 85 healthy controls and 40 healthy relatives were recruited. The samples were sequenced on the IonPROTON. The sequencing panel comprised of 187 genes, including *SHANK1* and *SHANK2* genes.Only samples with coverage of at least 95% were included in the analyses.SIFT&PolyPhen2 were used to identify pathogenic variants. The identified rare variants within *SHANK1* and *SHANK2* genes were validated by Sanger sequencing and tested in group of 100 patients and 100 controls.

Altogether 17 rare variants in *SHANK1* and *SHANK2*, present in affected only were found.Among the 16missense variants, 12 were potentially damaging. One splice variant was detected. Two of the missense variants in *SHANK2*, rs117843717 and rs150857128, were found both in schizophrenia and BAD patients. In the mutation burden analysis, *SHANK1* was one of the genes with more variants than expected in the BAD patients compared to controls.

SHANK1 and *SHANK2* mutations have been previously implicated in neuropsychiatric disorders. Recent functional analysis of *SHANK2* mutations found in schizophrenia patients support their causative role. The current data adds to the evidence that postsynaptic plasticity is involved in the pathogenesis of psychiatric disorder. The work was supported by projects DUNK01-2/2009 and D-131/2018MU-Sofia

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P09.107B
Identification of New Genes Associated with Childhood-Onset Schizophrenia, ATP1A3 and the FXYD Gene Family

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Childhood-onset schizophrenia (COS) is a rare and severe form of schizophrenia starting before age of 13. We identified two unrelated cases diagnosed with both COS and alternating hemiplegia of childhood (AHC), a rare disease characterized by repeated episodes of hemiplegia. Genes associated with AHC are known and guided our genetic exploration of these two patients using the targeted sequencing of the ATP1A3 gene. Then, we replicated our findings in a database of whole exome sequencing of 17 independent COS cases. In the cases with comorbid AHC, two distinct pathogenic de novo variants were identified in the ATP1A3 gene. In the replication cohort, we identified a third case with a possibly damaging missense variant in the same exon of ATP1A3. Three other cases with predicted pathogenic missense variants in the FXYD gene family (FXYD1, FXYD6, and FXYD6-FXYD2 readthrough) were found. ATP1A3 encodes the α-subunit of a neuron-specific ATP-dependent transmembrane sodium-potassium pump. The function of this pump is modulated by proteins encoded by the FXYD genes. Our report is the first to identify variants in the same pathway for COS. It illustrates the interest of exploring medical comorbidities and stratifying a complex condition according to the age of onset for the identification of deleterious missense variants. Whereas ATP1A3 is a replicated gene in rare neuropediatric diseases, we extended the phenotype to a pure psychiatric presentation. Our study highlights the interest of DNA sequencing in psychiatry and opens the way to develop genetic counseling in COS.

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P09.108C
Transcriptome analysis of post-mortem brain tissue reveals up-regulation of immune response genes in a subset of schizophrenia patients

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Introduction: Schizophrenia is a genetically complex neuropsychiatric disorder. While the heritability of schizophrenia is around 80%, the precise underlying molecular mechanisms and pathways are still unclear. To investigate pathways associated with schizophrenia, we performed gene expression study and we are now following up by measurement of protein expression.

Materials and Methods: Whole transcriptome poly-A selected paired-end RNA sequencing was performed on post-mortem brain tissue samples from 68 schizophrenia patients and 44 matched controls. Differential RNA expression was detected between cases and controls. The results were validated with RT-qPCR. Expression analysis for >100 proteins is currently under way using proximity extension assay.

Results: Significant differential expression was found for 71 genes in cases compared to unaffected controls. Gene ontology of differentially expressed genes revealed an up-regulation of multiple genes in immune response among the patients as the most significant category. Several genes of complement system, including *C1R, C1S, C7*, *FCN3* and *SERPING1* were also in the category. The increased complement expression was stronger in a subgroup of patients which might be due to differences in disease etiology within patients. Specific proteins were also associated with differential expression in this subgroup of patients. Weighted gene co-expression network analysis highlighted networks associated with synaptic transmission and activation of immune response.

Conclusions: Our results implicate upregulation of the complement cascade as a crucial pathway associated with schizophrenia pathology.

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P09.109D

SCN8A-related epilepsies: phenotypic overview and correlation with antiepileptic treatment

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Introduction: *SCN8A* is well-known gene being the monogenic cause of *SCN8A*-related developmental and epileptic encephalopathy, as well as self-limiting epilepsies and epilepsies with an intermediate phenotype, with mild intellectual disability and treatable seizures. Reports so far, suggest that sodium channel blockers (SCBs) have a positive effect on seizure in *SCN8A*-related epilepsy. In this overview, we sought to describe the complete phenotypic spectrum, provide novel data on functional studies, as well as evaluate treatment response in the patients suffering from epilepsy.

Methods: Clinical information on cognitive status, epilepsy onset, epilepsy type and treatment response was collected via standardized spreadsheets. Clinicians and geneticists worldwide participated in the study. Previously published cases were included via a PubMed search. All patients signed informed consent, and the study was approved by the local ethical committee.

Results: In total, we investigated 36 previously unpublished patients, and 246 patients in total. We found five distinct clinical subgroups; BFNIS, intermediate epilepsy, DEE, severe DEE (non-ambulant, no speech, cortical blindness) and patients without epilepsy. The DEE subgroups comprise 74% of the total cohort. SCBs were the most efficient anti-epileptic drug. Several recurrent variants were found. Some of these variants showed a persistent phenotype and others not. The underlying reasons for this remain unexplained.

Conclusion: The phenotypic range of *SCN8A* is wide ranging from self-limiting epilepsies to severe DEEs, and also including patients without epilepsy. Phenotypic variability within the same genetic variants are common, so genetic counselling must be done carefully. SCBs should be first-line treatment in *SCN8A*-related epilepsy.

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Severe neurodevelopmental disorder with intractable seizures due to a novel *SLC1A4* homozygous mutation

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Introduction: Biallelic mutations in the *SLC1A4* gene have been identified as a very rare cause of neurodevelopmental disorders with or without epilepsy and almost exclusively described in the Ashkenazi-Jewish population. That is why the *SLC1A4* gene is not routinely included in the genetic diagnostic panels for neurological diseases or epilepsy. Patients and Methods: Here we present a 5-year-old Czech boy with microcephaly, severe psychomotor retardation and intractable seizures who remained unsolved using clinical experience and standard diagnostic methods including examination by an epilepsy targeted gene panel. Whole-exome sequencing (WES) was finally used to obtain genetic diagnosis.

Results: A novel mutation, p.Arg457Gln (c.1370G>A) of the *SLC1A4* gene, was detected in homozygous state by WES in the patient, and afterwards by Sanger sequencing in heterozygous state in both parents confirming the biallelic origin of the mutation. This variant is not reported in ExAC/gnomAD databases in homozygous state, in heterozygous state in a very low frequency (0.002%), it affects a highly conserved aminoacid predicted to be deleterious. Mutation in the same codon but different aminoacid exchange was previously described in a patient with very similar phenotype, however, without epilepsy. Our patient suffers from microcephaly, global developmental delay, axial hypotonia with acral spasticity, brain and corpus callosum atrophy, impaired visual function and drug-resistant epilepsy.

Conclusion: Our data suggest that the *SLC1A4* gene should be considered in the diagnosis of patients with severe early onset neurodevelopmental impairment with epilepsy and encourages *SLC1A4* gene variants' analysis

via targeted gene panel or WES. Supported by: NV19-08-00194

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Molecular genetic and cellular characterization of a missense mutation in *SLITRK1* associated with non-syndromic autosomal recessive intellectual disability

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Introduction: Intellectual disability is a phenotypically and genetically heterogeneous neurodevelopmental disorder with an estimated prevalence of 1 % in the general population. So far sixty-nine gene loci have been reported to be involved in autosomal recessive non-syndromic intellectual disability (NS-ARID).

Materials and Methods: A novel disease locus was identified using homozygosity by descent (HBD) mapping coupled with whole exome sequencing in a large consanguineous Pakistani family. On the cellular level, the impact of the novel mutation on protein expression was characterized in subcellular colocalization experiments.

Results: Herein this study, HBD mapping identified a single shared homozygous region on chromosome 13 between markers rs9531043 to rs7318889. The subsequent exome sequence analysis identified a novel *SLITRK1* gene mutation, which segregated with the disease phenotype. SLITRK1 is an adhesion molecule located in the post-synaptic membrane of excitatory synapses. Cellular assays showed that the identified novel mutation results in loss of protein function by trapping mutated SLITRK1 intracellularly in the endoplasmic reticulum and thus preventing surface transport and ligand-binding. Additionally, several reported mutations showing decreased surface expression of SLITRK1 were selected for colocalization analysis. We proof that all variants are transported into the plasma membrane with the exception of *SLITRK1* harboring the novel mutation.

Conclusions: SLITRK1 has previously been associated with a variety of neuropsychiatric disorders. However, our findings suggest a possible, to date unreported, association of SLITRK1 in NS-ARID. Thus, in the light of our findings, the gene's implication in complex neuropsychiatric disorders should be re-evaluated.

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P09.112C

Somatic mutation that affects transcription factor binding upstream of *CD55* in the temporal cortex of a late-onset Alzheimer disease patient

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Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide. Familial cases suggest genetic components, however monogenetic causes are few and the vast majority of incidences have unknown cause. Sequencing efforts have focused on germline mutations, but improved technology has opened up for studies on somatic mutations in affected brain tissue samples. Here we use ultra-deep sequencing on brain and blood from early and late onset AD patients, and non-AD individuals (n=16). In total 2.86 Megabases of genomic regions, previously associated with AD was targeted, included 28 genes and upstream and downstream regulatory regions. Tailored downstream bioinformatics filtering identified 11 somatic single nucleotide variants in the temporal cortex in AD patients and none in the controls. One variant was validated to be present at 0.4% allele frequency in temporal cortex of a late-onset AD patient. This variant was predicted to affect transcription factor binding sites upstream of the *CD55* gene, contributing to AD pathogenesis by affecting the complement system. Our results suggest that future studies targeting larger portions of the genome for somatic mutation analysis are important to obtain an increased understanding for the molecular basis of both early and late onset AD.

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***Ddhd1*knockout mouse as a model for familial spastic paraplegia**

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We have previously identified a homozygous 4-bp deletion, in exon2 in *DDHD1*as the responsible variant for spastic paraplegia type 28 (SPG28) (Miura et al 2016). The variant is expected to cause a premature termination resulting in a functionally null allele. *DDHD1*encodes phospholipase A_1 (PA-PLA_1), an enzyme catalyzing phosphatidylinositol (PI) to lysophosphatidylinositol (LPI). Towards the clarification of the pathogenic mechanism of SPG28, we established *Ddhd1*knockout (KO) mice carrying a 1-bp deletion in exon 2 in *Ddhd1*, resulting in a frameshift and premature termination at the very similar position with the patient. Since we observed no obvious abnormalities in their locomotion, we measured foot-base-angle (FBA) which is an established character to evaluate SPG phenotypes. We observed a significant decrease of FBA in the homozygous KOs, as a partial replication of SPG phenotypes. By RNA sequencing, we identified 22 genes showing significantly changed expressions in cerebrum of the homozygous KOs. The 22 genes are characterized by a GO term, "positive regulation of GTPase activity" and a KEGG pathway, "NF-kappa B signaling pathway". By lipidome analyses using supercritical fluid chromatography mass spectrometry (SFC/MS) we observed significant increase of PI and significant decrease of LPI in cerebrum of the homozygous KOs. LPI is known to be the agonist for a G protein coupled receptor, GPR55 triggering mobilization of intracellular Ca^{2+}. Our current data suggest the mechanism of SPG that the reduced LPI level in cerebrum triggers abnormal reduction of intracellular Ca^{2+}, resulting in neural apoptosis by abnormal NF-kappa B signaling.

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Blood-based gene expression analysis in children with specific language impairment

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Introduction: Comparative gene expression profiling discovers differentially expressed genes associated with various diseases, including neurodevelopment disorders. Specific language impairment (SLI) is defined as an unexpected and persistent impairment in language ability despite adequate social environment and intelligence and in the absence of any explanatory medical conditions. This condition is highly heritable and affects around 7% of preschool children.

Materials and Methods: The study aimed to examine gene expression profiling in the peripheral blood of 60 individuals divided into two groups: children with SLI and age- and gender-matched healthy controls. A genome-wide sequencing of copy DNA molecules was conducted in the pooled probes of the two groups investigating the quantitative expression of the genes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway-based analysis was used to further understand genes' biological functions.

Results: As a result of the conducted expression analysis, 60 differentially expressed genes and 7 KEGG signaling pathways with statistical significant change were identified. The KEGG names of these pathways were: "Hepatitis C", "Herpes simplex infection", "Leukocyte transendothelial migration", "Primary immunodeficiency", "Calcium signaling pathway" and "Tight junction".

Conclusions: Clear and significant abnormalities exist in the gene expression in peripheral blood samples of children with SLI compared to healthy controls. We suggest that not only cellular signaling but immune mechanisms may also be involved in the pathogenesis of SLI.

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Overview of molecular diagnostic testing of spinocerebellar ataxias in Estonia

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Spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders. Due to extensive clinical overlap among different forms of hereditary ataxia (>35 subtypes known), genetic testing is required to establish a diagnosis. The most common causes of SCAs are expansions of trinucleotide CAG repeats within genes *ATXN1, ATXN2, ATXN3, ATXN7* and *CACNA1A*. Next-generation sequencing (NGS) assays have abled to delineate other types of hereditary ataxias caused by other variant types. **The aim of this study** was to clarify which types of SCAs are most prevalent in Estonia and to compare the diagnostic yield of trinucleotide repeat assay and (NGS) panel.

Patients and methods: The SCA repeat expansion assay was performed in 317 Estonian patients referred to the Tartu University Hospital during the years 2000-2018. All individuals were tested for triplet expansions in *ATXN1, ATXN2, ATXN3, CACNA1A* genes with PCR based fragment analysis, and since 2012 *ATXN7* was added to the standard panel. Since 2016, 72 patients were studied using NGS based panel (Illumina TruSight One) with ataxia subpanel (151 genes included).

Results: In 35 (11.0%) patients, repeat extension (34 SCA2 cases, 1 SCA1 case) was detected. From NGS-based panel testing, 7 (9.7%) diagnostic findings were reported in 6 different genes (*PRKCG, TGM6, ITPR1, SACS*, 2x *ANO10*, and *XPA*).

Conclusion: The most prevalent form of SCAs in Estonia is SCA2. Triplet repeat expansion assay and NGS panel sequencing showed similar diagnostic yields, but it is more cost-efficient to start with repeat expansion assay. Funding: Estonian Research Council grants PRG471, and PUTJD827.

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Identification of genetic factors that modify age at onset of spinocerebellar ataxia type 3

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Introduction: There is a strong correlation between CAG repeat size of *ATXN3* and the age at onset (AAO) of spinocerebellar ataxia type-3 (SCA3). However, AAO variability is not entirely explained by the length of the repeat, suggesting the existence of additional modifying factors.

Materials and Methods: To examine the residual variability in AAO, a genome-wide association study (GWAS) was conducted across a cohort of 700 SCA3 cases from North America, Brazil and Portugal. The first phase of the study established the CAG repeat size in cases as it assessed the proportion of AAO variability that could be accounted to the repeat size. In the second phase, a linear regression analysis tested for genomic factors affecting the residual AAO.

Results: The inverse relationship between CAG repeat size and AAO was confirmed ($\rho=0.62$). No loci reached genome-wide significance but eight suggestive loci ($p<1\times10^{-5}$). We also observed 80 loci with a $p<1\times10^{-4}$; some of which revealed associations with genes causing other forms of ataxia. The later would be good candidates for the AAO of SCA3.

Conclusions: This is the first GWAS to focus on the identification of variants that could reveal modifiers of AAO in SCA3. We confirmed the known inverse correlation between CAG repeat size and AAO as we provided evidence for other modifying factors. A replication study using a well-powered independent cohort is now required to confirm our results.

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Distance between oculomotor patterns in premanifest spinocerebellar ataxias reflects disease onset

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Introduction: Knowledge about premanifest stage of spinocerebellar ataxias (SCAs) will guide therapeutic preventive interventions. The aim of this work was to follow oculomotor and cognitive changes over time in unaffected premanifest SCAs carriers.

Materials and Methods: As part of the RISCA study, we assessed preclinical SCA1-2-3 carriers and non-carriers at baseline and at 72 months follow-up. Distance between Oculomotor Patterns (DOP) were determined by combining values of standard deviation from the norm of prosaccades latency, velocity and gain for leftward and rightward prosaccade, antisaccade latencies and square-wave jerks (pathological if >4) and correlated with cognitive outcomes. Quantitative motor battery and volumetric MRI were performed.

Results: There were 16 carriers (3 SCA1 carriers, 4 SCA2 carriers, 9 SCA3 carriers) and 16 non-carriers, similar in age (mean age at baseline 35.7±7.8), quantitative cerebellar functional score (CCFS), cognitive evaluation (MMS, BREF and Stroop) and DOP at the baseline. Interestingly, SARA score at baseline was below five but higher in carriers (1.47±0.85 vs 0.78±0.72, $p<0.05$). At follow-up, DOP increased from 2.2±1.7 to 2.2±3.8 in non-carriers and from 3.5±3.6 to 8.2±8.2 in carriers ($p<0.05$). For the converters with SARA>5, mean DOP reached 12.5±8.2 while it remained at 3.3±5.3 in non-converters ($p=<0.01$). There was a positive correlation between DOP and CCFS ($p=0.01$) and SARA ($p=0.001$). No changes in cognitive assessments were found at follow-up.

Conclusions: DOP and CCFS reflect disease conversion and are independent from the rater thus more reliable in presymptomatic carriers.

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P09.118A

Novel glycine receptor variants identified in startle disease patients

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Startle disease, also known as hyperekplexia, is a rare inherited neurological disorder that affects newborn children. The predominant genetic causes are mutations in the genes*GLRA1* and *GLRB*, encoding glycine receptor alpha and beta subunits, respectively, and *SLC6A5* encoding the glycine transporter GlyT2. The disease has hallmark characteristics of noise- or touch-induced non-epileptic seizures resulting in muscle stiffness and apnea. However,

cases are often misdiagnosed as spastic quadriplegia or epilepsy. Our study focuses on likely disease causative mutations in startle disease patients in the Kingdom of Saudi Arabia. Sanger sequencing, homozygosity mapping, and targeted next-generation sequencing were conducted in fifteen patients. Functional analysis of selected variants was performed using whole-cell recordings and confocal microscopy in acutely transfected N2A cells. We identified one novel (p.A455P) and two (p.Q195X and p. M177R) recurrent variants in the *GLRB*. We also identified one novel frameshift mutation in the *SLC6A5*(p.I692fs) and three recurrent variants in *GLRA1* (p.G342S, p.R252H, and p.R218Q). Cells transfected with mutant *GLRB* showed a reduction in glycine-evoked currents compared to those of wild type. Tagging of glycine receptor subunits further revealed disrupted membrane localisation of the mutated proteins. Our results uncover novel mutations in glycine receptor genes that are potentially linked to the aetiology of startle disease

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P09.119B

Characterization of*STUB1*in zebrafish - development of a new knock-out model to study neurodegeneration

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Introduction: Autosomal Recessive Spinocerebellar Ataxia-16 (SCAR16) is caused by deficiencies in the *STUB1* gene encoding the ubiquitin E3 ligase and co-chaperone CHIP. There is limited knowledge regarding the pathogenic role of mutant CHIP *in vivo*. This study aims to establish a zebrafish model for SCAR16 by CRISPR/Cas9-mediated mutagenesis of the zebrafish orthologue of human *STUB1*.

Material and Methods: Single guide RNAs targeting a 20 nucleotide-sequence in *stub1* gene were injected together with Cas9 proteins into wild type zebrafish embryos at one-cell stage. Offspring fish were analyzed for transmitted germline mutations. The expression of CHIP protein was also examined in WT fish brain using immunohistochemistry.

Results: All injected eggs showed high frequency of modifications at the *stub1 locus* at 48 hours post injection. F1 heterozygous carriers with a 7 nucleotide-deletions at position 10800 in the *stub1* gene were produced from the founder fish, and further used to obtain homozygous offspring. Immunohistochemistry studies provided initial evidences regarding localization of CHIP protein in Purkinje cells in the cerebellum of WT fish.

Conclusions: We here described the use of CRISPR/Cas9 system to induce modifications of the *stub1* gene in zebrafish and contribute to establishing a new animal model for studying *STUB1*-related ataxia diseases. Future work will focus on phenotypic and behavior analysis of homozygous mutant zebrafish lines.

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P09.120C

Proposal for a new subcategory in the classification of the Copy Number Variants as Factor of susceptibility

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Introduction: The interpretation of variants of uncertain significance (VOUS) remains a challenge for geneticists since the criteria used and the continuous updating of the databases may change their classification. We reviewed the postnatal recurrent CNV in neurodevelopmental disorders with lack of agreement in the interpretation across databases and labelled as VOUS, probably pathogenic or probably benign with the aim of reclassifying them as a susceptibility factor.

Materials and Methods: A CGH array (8x60K ISCA, Agilent Technologies) was performed on 2,858 patients with neurodevelopmental disorders. We define a susceptibility factor as a CNV with reduced penetrance, variable clinical expressivity and complex to associate with a specific phenotype.

Results: A total of 69 (2.4%) patients with a CNV classified as a susceptibility factor have been identified. Dup3p26.2 (*CNTN4* / *CNTN6*) in 7 cases, del15q11.2

(*NIPA1*) in 22 cases, dup15q13.3 (*CHNRNA7-OTUD7A*) in 17 cases, dup16p13.11 (*NDE1, NTAN1*) in 5 cases, dup16p13.3 (*RBFOX1*) in 3 cases and dupXp22.33 (*SHOX1*) in 16 cases. A second CNV was detected in 11 cases and was classified mostly as VOUS. The variable clinical expression observed in each of these variants agrees with what is described in the literature.

Conclusions: A new subcategory of susceptibility factor is proposed for these CNVs classified in the VOUS group. Its clinical significance is subject to the penetrance and expression in each individual. Given the wide heterogeneity, the clinical geneticist must decide if the CNV is responsible for the phenotype and determine if the family segregation study is necessary.

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P09.121D

Expanding the "Angelman-like" spectrum: patients with SYNGAP1-related disorders show EEG findings most often attributed to Angelman syndrome

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Introduction: The group of Angelman-like neurodevelopmental syndromes continues to expand. In patients with characteristic behaviors, developmental delays, speech impairment and seizures, specific EEG patterns have been proposed as having relative specificity for Angelman. Here we report on two patients with Angelman-associated phenotypes and EEG findings who were ultimately diagnosed with SYNGAP1 syndrome.

Materials and Methods: Patient 1 is a female with infantile onset oropharyngeal dysphagia, hypotonia, global developmental delays, and early childhood epilepsy. EEG findings appeared to suggest Angelman syndrome, however, targeted testing was negative. Further molecular testing confirmed a pathogenic variant in SYNGAP1.

Patient 2 is a male with similar history of oropharyngeal dysphagia, hypotonia, global developmental delays, and childhood onset epilepsy in addition to tremors and ataxia. EEG findings showed patterns suggestive of Angelman, with a final diagnosis of SYNGAP1 syndrome.

Results: Patient 1 EEG: rhythmic posterior delta slowing with embedded spikes, generalized epileptiform activity

Patient 1 molecular testing (via Whole Exome Sequencing): De Novo, SYNGAP1 p.Q1106X

Patient 2 EEG: Rhythmic posterior notched delta slowing increased with eye closure, generalized and left central focal epileptiform activity

Patient 2 molecular testing (via Whole Exome Sequencing): De Novo, SYNGAP1 p.R967X

Conclusions: Given the degree of clinical and EEG finding overlap, SYNGAP1 syndrome should be considered in patients with an Angelman-like phenotype.

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P09.122A

Familial Adult Myoclonic Epilepsy linked to chromosome 5p15 (FAME3) is caused by an intronic ATTTT/ATTTC expansion in *MARCH6*

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Introduction: Familial Adult Myoclonic Epilepsy (FAME) is an autosomal dominant disorder characterized by cortical or myoclonic tremor occurring in association with generalized seizures typically beginning in adulthood. Despite the early identification of multiple loci on chromosomes 8, 2 and 5, the genetic basis of this condition has remained elusive for 20 years. Recently, intronic expansions of ATTTT/ATTTC pentanucleotide repeats in *SAMD12* on chromosome 8 have been identified as a major cause of FAME in Asia.

Methods and Results: In this study, we used genome sequencing (Illumina, short-read technology) and repeat-primed PCR to identify a new expanded site in *MARCH6* on chromosome 5p15 in four European families. Further analysis of single DNA molecules using long-read nanopore sequencing and, in parallel, molecular combing combined with fluorescent staining of the ATTTC repeats and flanking regions, revealed that the expansions range on average from 4 to 13 kb. However, we observed a high variability in expansion length and structure, compatible with the existence of different expansion configurations in the blood cells of the same individual. Moreover, the largest expansions were associated with complex microrearrangements occurring at the expanded site in up to 20% of the cells.

Conclusion: This study provides further evidence that FAME is homogeneously caused by intronic ATTTT/ATTTC expansions in distinct genes and shows that expansions exhibit a high somatic instability that can ultimately result in genomic rearrangements.

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P09.123B

A significant inflation inTGM6genetic risk casts doubt in its causation in spinocerebellar ataxia type 35

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An autosomal dominant type of spinocerebellar ataxia (SCA), SCA35, has been associated with pathogenic mutations in the gene TGM6. In a Chinese exome sequencing cohort with 116 indexes and 135 asymptomatic parents, we identified 8 families with reported TGM6 variants sharing no features of SCA35. Considering this finding, we reviewed the public database gnomAD, these reported pathogenic variants are significantly more commonly found in the East Asians than in other ethnic groups ($P<0.0001$). Bioinformatics analysis of gene constraint showed that both missense and loss-of-function variants in TGM6 are likely to be tolerated with no regional constraint. By performing inflation analysis to empirically evaluate the pathogenicity of TGM6, it is found that there is an over-representation of reported pathogenic variants in the population. The cumulative frequency of reported pathogenic TGM6 variants is at least 111-fold inflated over the combined prevalence of all autosomal dominant SCAs (<5.6 per 100,000), indicating a high chance of misdiagnosis or very low penetrance. Misclassification of benign or low penetrant variants as pathogenic is a significant problem that often results in genetic misdiagnosis and premature ending of diagnostic odyssey. This highlights the necessity of evaluating variant pathogenicity with sequencing of genomes from diverse populations, both from asymptomatic controls and phenotypically different patients, in order to ensure accurate classification of variants.

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P09.124C

Autosomal recessive tubulinopathy-like disorder caused by loss of function of the tubulin-modifying enzyme *Tubulin Tyrosine Ligase*

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Introduction: Tubulin Tyrosine Ligase (TTL) is a ubiquitous enzyme that catalyzes the convertion of detyrosinated to tyrosinated α-tubulin, a process that regulates multiple essential microtubule functions. Deletion of TTL in mice causes perinatal death due to a strong accumulation of detyrosinated α-tubulin in the brain, which leads to massive aberrations in brain development. Moreover, TTL has recently been demonstrated to be crucial for post-injury axon regeneration.

Materials and Methods: We describe two sisters from healthy consanguineous parents, presenting with global developmental delay, intellectual disability, cerebellar ataxia, hypotonia and microcephaly. Brain MRI findings was suggestive of a tubulinopathy, showing dysmorphic basal ganglia, commissural agenesis or hypoplasia, dysplasia of the cerebellar vermis and brainstem. After excluding mutations in tubulin genes, whole exome sequencing analysis was performed.

Results: Both siblings shared the homozygous missense variant c.1013G>A (Cys338Thr) in the *TTL* gene (NM_153712.4). The variant was predicted as pathogenic by all prediction software and was absent from population databases. Molecular dynamics simulation demonstrated that the mutant protein is more flexible than wild type, which could lead to impaired interaction with α-tubulin, and thus, impaired enzymatic function. Indeed, enzymatic activity assays showed that mutant TTL protein is inactive, while patient fibroblasts show significantly higher levels of detyrosinated α-tubulin compared to healthy controls.

Conclusion: We report the first recessively inherited tubulinopathy-like disorder, caused by biallelic loss of function mutations in the TTL gene. Funding: ERC Starting Grant 260888; Ricerca Finalizzata NET-2013-02356160

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P09.125D

Reduced VPS13C protein expression caused by recessive missense and splice site mutations is associated with Lewy body disease and Parkinson disease

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Introduction: Lewy body disease (LBD) and Parkinson Disease (PD) share clinical and pathological features. *VPS13C* was identified as a risk factor for PD in GWAS, and premature termination codon (PTC) mutations in *VPS13C* were associated with recessive PD. We identified in whole genome sequencing data of a recessive early-onset age LBD family (AAO: 41-42 years) compound heterozygous *VPS13C* missense mutations reducing VPS13C protein expression.

Materials and Methods: Targeted resequencing of coding *VPS13C* in 230 LBD patients (AAO: 70.8±9.7 years), 612 PD patients (AAO: 60.5±11.1 years) and 663 control individuals (AAI: 72.0±9.4 years). Protein analysis was performed in lymphoblast cells and brain lysates of LBD compound heterozygous *VPS13C* mutations carriers.

Results: We identified 4 LBD carries of compound heterozygous missense mutations in *VPS13C*. Two mutant alleles decreased protein expression by near 90% in lymphoblast cells. VPS13C reduction was also observed in autopsy brain of two LBD carriers. In the PD cohort, we identified 7 patients compound heterozygous (splice site/missense or missense/missense) and 1 patient homozygous (missense) for *VPS13C* mutations. In control individuals, we observed 6 carriers of compound heterozygous mutations of which 3 shared the same variants suggesting *cis* configuration. *Trans*/*cis* position of the heterozygous *VPS13C* alleles are being determined by long-read sequencing on *VPS13C* cDNA.

Conclusion: Our genetic and expression data suggest that also *VPS13C* missense mutations contribute to risk for LBD or PD by loss-of-function mechanism. Additional functional studies will be needed to understand the contribution of the different mutated *VPS13C* alleles to LBD and PD.

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P09.126A

Comprehensive rare variant analysis of individuals with neurodevelopmental disorders by whole-genome sequencing

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Despite the significant progress in unravelling the origins of neurodevelopmental disorders (NDDs), up to 50% of affected individuals remain without a genetic diagnosis. Here we demonstrate the power of using whole-genome sequencing (WGS) to identify pathogenic variants in NDDs, in one of the largest studies to date. WGS was performed on 691 individuals (488 affected and 201 unaffected relatives). Initially, pathogenic variants were identified in 31% individuals, and 21% had a variant of uncertain significance. Reanalysis of the data two years later increased the yield to 36% and 23% respectively. Of all reported variants, 90% were SNVs/indels in coding regions of NDD-associated genes. However, WGS provided power to detect three additional categories of variants: structural, intronic and mitochondrial variants. Structural variants (SVs) comprised 25 deletions, 6 duplications, 2 inversions, one partial uniparental isodisomy and one complex SV. Nanopore long-read WGS was used to resolve the genetic architecture of SVs that could not be resolved by other methods. Furthermore, 7 pathogenic intronic variants were identified, as well as 3 likely pathogenic variants in the mitochondrial genome. This study demonstrates the value of using WGS to investigate the genetic causes of NDD as a single pass investigation. It also corroborates previous reports that reanalysis of data allows more patients to receive genetic diagnoses. WGS provides comprehensive and unbiased coverage facilitating identification of all types of variants throughout the human nuclear and mitochondrial genome. Importantly, this includes variants that would not have been identified using the more commonly used whole exome sequence approach.

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P09.127B

***De novo*, heterozygous missense variants in *YWHAG* as a novel cause of developmental and epileptic encephalopathy**

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Introduction: Developmental and Epileptic encephalopathies (DEE) describe heterogeneous epilepsy syndromes, characterised by early-onset, refractory seizures and developmental delay (DD). With increased access to whole exome sequencing (WES), new candidate genes are being identified. *YWHAG* (*605356) on Chr 7q11.23 encodes for YWHAG, from the 14-3-3 protein family, playing important roles in signal transduction and cellular proliferation. YWHAG is highly expressed in brain, skeletal and heart muscle. Mouse models suggest alterations in Ywhag level cause delayed neuronal migration and interstitial deletions at Chr 7q11.23 result in infantile seizures and cardiomyopathy, suggesting haploinsufficiency as an explanation.

Materials and Methods: We present 12 patients with *YWHAG de novo* missense variants. We describe a syndromal phenotype, report novel and a recurrent p.Arg132-Cys *YWHAG* variant and compare developmental trajectory and treatment strategies in this cohort. Patients 1-9 had WES via Deciphering Developmental Disorders Study. Patients 10-12 were identified via Genematcher and AnnEX databases. *De novo* variants from exome data were validated using Sanger sequencing.

Results: 12/12 patients in the cohort have *de novo*, heterozygous missense variants in *YWHAG* including 4/12 patients with a recurrent c.394C >T, p.Arg132Cys variant. Characteristic features included: early-onset seizures, predominantly generalised tonic-clonic and absence type with good response to standard anti-epileptic medications; DD; Intellectual Disability (ID) and a recognisable facial gestalt.

Conclusions: Our findings support the hypothesis that *YWHAG* loss-of-function causes a neurological phenotype. Although the exact mechanism of YWHAG is not fully known, it is likely that YWHAG haploinsufficiency in developing cerebral cortex leads to abnormal neuronal migration resulting in DEE.

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P10
Neuromuscular disorders

P10.01D
Analysis of miRNA promoter methylation profiles in ALS patients

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Introduction: Many micro RNAs (miRNAs) are aberrantly expressed in ALS patients as a result of interrupted miRNA biogenesis and processing or due to other changes that affect miRNA expression levels, including DNA methylation. Several studies showed global methylation changes in ALS patients that could lead to altered expression levels. However, none of them focused on the influence of promoter methylation on miRNA expression.

Materials and Methods: We identified methylated regions in the genome of ALS patients through MeDIP sequencing (methylated DNA immunoprecipitation and sequencing of precipitated DNA fragments).

Results: Sequenced fragments were mapped to a total of 1321 miRNA promoters. Based on the methylation differences between samples we selected 358 of them for further assessment. Using KEGG analysis it turned out that miRNAs with altered methylation status in promoter region target numerous genes including those involved in ErbB signalling pathway ($p= 1*10^{-8}$), Hippo signalling pathway ($p=2*10^{-8}$), prion diseases ($p=9*10^{-6}$), long-term depression ($p=9*10^{-6}$), endocytosis ($p=2*10^{-5}$), adherens junctions ($p=3*10^{-5}$), and axon guidance ($p=4*10^{-5}$).

Conclusions: Promoter methylation of miRNA genes could be an important factor for their aberrant expression in ALS. Since miRNA target genes are involved in diverse biochemical pathways and processes, aberrant expression of miRNAs may contribute to ALS pathology. However, further validation studies are needed to confirm this.

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P10.02A
Comprehensive genetic analysis in a cohort of 107 Hungarian patients with amyotrophic lateral sclerosis

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the degeneration of upper and lower motor neurons. The frequency of mutations in ALS patients has been extensively investigated in populations of various ethnic origins. Patients and methods: All investigated ALS patients (n=107) were of Hungarian origin. Repeat sizing of *C9orf72* and *ATXN2* genes and targeted next generation sequencing (NGS) were used to comprehensively assess genetic variation in 35 major ALS genes. The cohort was previously screened for *SOD1* and *TARDBP* genes, with negative results. Variants from whole-exome sequencing data of other studies (200 individuals of Hungarian or Austrian origin) were used as in-house control database.

Results: Pathogenic repeat expansions in the *C9orf72* gene were detected in 10 patients (9.3%); intermediate polyQ lengths (27-33) in the *ATXN2* gene were identified in 9 (8.4%) patients. Using panel sequencing, 31 rare, potentially pathogenic variants were detected in 16 genes. According to the NGS results, the most frequently mutated genes were *NEK1* (5.6%), *NEFH*, *SQSTM1* (3.7%), *KIF5A, SPG11* (2.8%), *ALS2, CCNF, FUS, MATR3, TBK1* and *UBQLN2* (1.9%). Furthermore, potentially pathogenic variants were found in *ERBB4, FIG4, GRN* and *SIGMAR1* genes in single patients.

Conclusion: Combining targeted NGS and repeat sizing, potentially causative variants were detected in 41% of patients including patients with variants considered to be of uncertain significance (VUS). Our findings highlight the necessity for large-scale multi-center studies on ALS patients to better understand the underlying genetic causes. Funding: Hungarian Brain Research Program (Grant No. 2017-1.2.1-NKP-2017-00002).

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P10.03B
Bringing genetics into clinical practice: antisense oligonucleotide (ASO) therapy in 5q-associated spinal muscular atrophy (SMA)

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Introduction: The neuromuscular disorder SMA is the most frequent genetic cause of death in children. Due to lower motor neuron loss caused by homozygous deletions in the survival of motor neuron (SMN) 1 gene patients suffer from progressive muscular atrophy and paralysis. Whereas SMA 1 and 2 patients never walk independently, SMA 3 patients present with a milder phenotype and later onset mainly attributable to higher copy numbers of the only partially functional SMN2 gene. In 2017 the intrathecally administered antisense oligonucleotide (ASO) Nusinersen has been approved as first treatment for all types of SMA by the EU, based on results from phase 3 trials in pediatric patients. Nusinersen alters the splicing of SMN2 pre-mRNA in order to increase production of full-length SMN protein. Long term evaluation of treatment effects and impact of SMN2 carrier status in adult SMA patients under ASO therapy is urgently needed.

Materials and Methods: In our center, 24 patients aged 18-65 years have been treated with intrathecal loading doses of Nusinersen at day 1, 14, 28 and 63, followed by maintenance doses every 4 months so far. Treatment efficacy was assessed by monitoring of muscular functions and patient reported outcome measures in correlation to the SMN2 carrier status.

Results: Intrathecal administration either by conventional or computed tomography (CT)-guided lumbar puncture was well tolerated. No patient has developed communicating hydrocephalus. First objective and subjective results indicate a benefit in motor function in dependence of disease severity and SMA type which correlates to the SMN2 copy number.

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P10.04C
A *de novo* mutation of the stress granules-associated CAPRIN1 causes a novel progressive neurodegenerative disorder

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Introduction: Motor neuron disorders (MNDs) are neurological diseases whose diagnosis is still challenging due to their highly overlapping phenotypes and genetic heterogeneity.

Materials and Methods: An eight year-old girl born from a non-consanguineous couple, presented a progressive motor-sensory axonal polyneuropathy and subsequently developed cerebellar ataxia, dysphagia and cognitive decline. She underwent *SMN1* deletion testing, NMD-gene panel analysis and a trio WES.

Results: WES led to the identification of a *de novo* c.1535C>T variant (p.Pro512Leu) in the *CAPRIN1* (Cell Cycle Associated Protein 1) gene. This variant is not present in 1000G and gnomAD. *CAPRIN1* has reduced tolerance to missense ($Z = 1.79$) and is intolerant to nonsense variants ($pLI = 1.0$). CAPRIN1 is abundant in the adult brain, where it regulates the transport and translation of mRNAs of genes involved in synaptic plasticity. Moreover, it contains an LC/ID domain and it is a component of stress granules. Mutations in proteins containing LC/ID domains (TDP43, TIA1, hnRNPA1/2) cause MNDs and increase the likelihood of protein aggregates formation. Several *in silico* tools predict that the p.P512L mutation to increase the aggregation propensity. Indeed, experiments in HEK293T cells show that $CAPRIN1^{P512L}$ forms bulky aggregates as compared to $CAPRIN1^{WT}$. Moreover, an insoluble protein extraction assay demonstrates that $CAPRIN1^{WT}$ elutes in the RIPA-soluble fraction while $CAPRIN1^{P512L}$ in the urea-soluble one.

Conclusions: Our results suggest that $CAPRIN1^{P512L}$ is prone to aggregation. As CAPRIN1 deficiency is linked to autism-spectrum disorders (ASD) in human and mice, we propose $CAPRIN1^{P512L}$ as a novel gain-of-function NMD candidate gene.

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P10.05D
Neuropathy related genes as possible modifiers of PMP22 related neuropathies

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Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP) are autosomal dominantly inherited peripheral neuropathies caused by copy number variation of the PMP22 gene. Considerable phenotypic variation is known for both disorders, suggesting modifiers.

We undertook a search for genetic modifiers of PMP22 related neuropathies by selecting the extremes of the spectrum of CMT1A and HNPP patients, based on disability assessed by the Overall Neuropathy Limitation Scale (ONLS). The ONLS data of 287 patients (184 CMT1A and 103 HNPP patients) showed a Gaussian distribution for both disorders. The median score for CMT1A patients was 4 and for HNPP patients 3. Twenty-one mild CMT1A (ONLS <2), 26 severe CMT1A (ONLS >5), 25 mild HNPP (ONLS <2) and 25 severe HNPP patients (ONLS >4) were clinically evaluated to further characterize disease severity. A next generation sequencing gene panel containing 147 genes related to neuropathies and hereditary motor syndromes was tested in this selection of patients.

Missense, frameshift, nonsense and intronic mutations possibly affecting splicing were selected. No significant difference was found in the mean number of variants (mutation burden) per patient between the mild and severe groups. Further selection of variants with an allele frequency of ≤4% in a control population was done. A significant difference between the mild and the severe HNPP group was found. In several patients double trouble was found (another likely pathogenic mutation in a neuropathy related gene). Replication of these results in a larger cohort are needed.

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P10.06A
A novel ddPCR method for the copy number variation analysis of the segmental duplication regions in nebulin and titin

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Introduction: The giant genes nebulin (*NEB*) and titin (*TTN*) both harbour large expressed segmental duplication (SD) regions. Mutations in *NEB* cause nemaline myopathy (NM), and mutations in *TTN* cause myopathies and cardiomyopathy. Using custom, targeted Comparative Genomic Hybridization arrays for NM and other neuromuscular disorders, we have shown that copy number variations (CNVs) in the *NEB* SD are potentially pathogenic. We have also shown that the *TTN* SD harbours recurrent and potentially pathogenic CNVs. For validation, we have established custom Digital Droplet PCR (ddPCR) assays targeting the SD regions of *NEB* and *TTN*.

Materials and Methods: We created custom assays for exons 4 and 8 of the *NEB* SD, and exon-intron regions 1-2 and 7-8 of the *TTN* SD. The ddPCR method was validated using 70 controls previously run on our CGH-arrays.

Results: We show that CNVs in the *TTN* SD are recurrent and appear in 25 % of both healthy and affected individuals. The ddPCR method is sensitive, and reliably detects CNV in both *NEB* and *TTN* SDs.

Conclusions: Our ddPCR assay for the SD regions of *NEB* and *TTN* allows for rapid, specific and inexpensive assessment of CNVs within these regions. Currently, we are investigating the potential pathogenicity of *TTN* SD region CNVs.

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P10.07B
Expectations and anxieties of Duchenne muscular dystrophy patients and their families during the first-in-human clinical trial of NS-065/NCNP-01

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Duchenne muscular dystrophy (DMD) is a recessive X-linked genetic disease caused by a mutation in the dystrophin gene. The new drug NS-065/NCNP-01 utilizing exon-skipping therapy targeting specific deletions has been used in a first-in-human trial for the treatment of DMD. We surveyed 10 pairs of DMD participants and their parents within this clinical trial via an iPad survey form and through interviews regarding their understanding of the trial, expectations, anxieties, and reasons for participating in the trial. Approximately half of the participants actively decided to participate of their own volition, and none considered quitting the trial. This indicates that participants participated more positively in this clinical trial than previously expected. However, some potential concerns were also revealed, with one being that the desire to please those around them might be more important to the DMD participants than the effects of the drug. Another issue is the possibility of biased information originating from the study subjects' parents; while seven out of 10 of the parents told their children that the study drug might work, only four of these parents also explained that it might not work. Only two study participants received an explanation concerning the drug's side effects from their parents. This result implies that caution should be taken when family expectations are high, and there is a possibility that subjects will be given biased information from their parents. This work was supported grant from the [Intramural Research Grant (26-6) for Neurological and Psychiatric Disorders of National Center of Neurology and Psychiatry]

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P10.08C
Genomic profiling of adult onset isolated focal dystonia in a group of Romanian patients

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Introduction: We proposed to identify the relevant genomic profiles of adult-onset isolated focal dystonia by gaining insights into the interplay of potential causative disease-associated genetic variants at the genomic level. Isolated focal dystonia is the third most common movement disorder after Parkinson's disease and essential tremor. The etiology is genetic in some cases, but over 75% are idiopathic.

Materials and Methods: In the first stage of our study, we analyzed onto the Ion Torrent PGM platform a well-described homogenous group of 120 Romanian dystonia cases using our own designed Targeted Sequencing AmpliSeq panel of 30 genes previously found to be associated with dystonia (DYT) and other movement disorders. All samples had 97.85% average of target regions with coverage by at least 30 folds. Data were analyzed using a bioinformatics in house pipeline based on GATK, Picard and Annovar software for reading alignments, variant calling, filtering and annotation.

Results: Molecular genetic screening identified mutations in 46/120 (38%) of the clinically diagnosed adult-onset isolated focal dystonia subjects. All identified mutations were previously reported to be associated with DYTs.

Conclusions: The subsequent analysis of negative-known DYTs patients for the genes in the customized panel using the Whole Exome Sequencing could offer the opportunity to assess extensively the genomic profiles of adult-onset idiopathic isolated focal dystonia and could result in identification of new genes and variants causing-disease. This work was supported by the CNCS-UEFISCDI grant PN-III-P4-ID-PCE-2016-0696.

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P10.09D
Cellular heterogeneity in FSHD

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Single-cell analyses have become increasingly important for uncovering cellular dynamics during health and disease. Not only have they began to unravel a greater level of cellular heterogeneity than previously could have been appreciated, it also enables the identification of sporadic cellular aberrations involved in disease etiology and development.

One prime example of disease-associated cellular heterogeneity is found in facioscapulohumeral muscular dystrophy (FSHD), which is characterized by sporadic de-repression of the cleavage-stage transcription factor DUX4 in ~1:200 myonuclei. Yet, with the sporadic nature of DUX4 de-repression limiting the resolution of bulk RNA-seq analyses, essential insights in the cascade of events leading to apoptosis and severe muscle wasting observed in patients are currently limited.

We used single-cell RNA-sequencing combined with pseudotime trajectory modeling to generate a detailed FSHD cellular progression model. We show that our model detects FSHD-associated cellular progression in approx. 1% of all cells, and faithfully reflects both the burst-like DUX4 expression as well as the downstream activation of various pathways previously implicated in FSHD. In addition, using our model we were now able to identify new expression signatures that are likely related to both direct and indirect effects of DUX4.

Our data therefore highlights the potential for single-cell transcriptomics in studying disease etiology in genetic diseases, even when hallmarked by high cellular heterogeneity as in FSHD, and shows that pseudotime trajectories like our FSHD pseudotime model may contain valuable information for further unraveling the role of cellular heterogeneity in disease etiology and progression.

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P10.10A
Functional analysis of an intronic variant of uncertain significance c.911-13A>G in the *FKTN* gene

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Introduction: Next generation sequencing methods allow the identification of previously ignored deep intronic variants, but it is still difficult to determine their

pathogenicity. In a patient with congenital muscular dystrophy and cardiomyopathy, two heterozygous variants in the *FKTN* gene were identified: previously described pathogenic variant NM_001079802.1(FKTN_v001): c.509C>A p.(Ala170Glu) and a previously undescribed intronic variant of uncertain significance NG_008754.1 (FKTN_v001): c.911-13A>G, inherited from the mother.

Materials and Methods: In order to study the effect of c.911-13A>G on *FKTN* splicing and confirm its pathogenicity, we performed RT-PCR of total RNA isolated from peripheral blood mononuclear cells and fibroblasts obtained from the proband, his mother and healthy donors, as well as using a *FKTN* minigene expression system in a HEK293T cell model line.

Results: When studying the obtained mRNA structure, three *FKTN* isoforms were identified: two normal (present in control samples of healthy donors) and one aberrant with exon 9 skipping. The minigene expression analysis confirmed this data. The short isoform can be explained by the destruction of the intron 8 acceptor splice-site. Thus, the work showed that the presence of a deep intronic variant c.911-13A>G in the *FKTN* gene leads to the loss of 133 nucleotides, with an open reading frame shifting and a 145 amino acid shortening (p.W301Rfs*13) of the protein.

Conclusions: Functional analysis of intron variants allows to establish the pathogenicity of variants of uncertain clinical significance and confirm the diagnosis in patients. In this study, the pathogenicity of the c.911-13A>G variant in the *FKTN* gene was demonstrated.

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P10.12C
Methods of the DNA diagnostics for facioscapulohumeral muscular dystrophy (FSHD)

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Introduction: In 95% FSHD caused by a partial contraction of the D4Z4 array up to 10 - 1 repeats at a 4qA haplotype of chromosome 4q35. The size of D4Z4 repeats and its high similarity with the D4Z4 locus on chromosome 10q26 makes diagnostic of FSHD complicated for basic diagnostic laboratories.

Materials and Methods: Blood-derived DNA was isolated from 55 FSHD patients and its relatives. For the molecular-genetic diagnostic, we used three methods: Southern blotting, molecular combing and a newly developed PCR based method.

Results: Totally 55 individuals were analyzed by the Southern blotting, among them 12 parents/children, 28 sporadic cases. Six patients with difficult interpretation of the Southern blotting results were further analyzed by molecular combing. For all cases we determined the D4Z4 repeat size and haplotype. The PCR based diagnostic was applied to 7 FSHD patients with one 4qA repeat below 10 units and to 8 healthy relatives with normal-sized D4Z4 repeats. The PCR results were concordant with the Southern blotting in 57.1% for the patients and in 88% for the healthy relatives. We suggest that the discordant results were obtained due to high background of amplification or to DNA quality issues.

Conclusions: The Southern blot and the molecular combing results are highly correlated. The PCR-based method shows potential to diagnose FSHD; however needs further optimization. Improvement of the PCR-based method may simplify FSHD diagnostic for basic diagnostic laboratories.

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P10.13D
Genetic analysis of autosomal dominant motor and sensory neuropathy with proximal dominancy in the lower extremities, urinary disturbance, and paroxysmal dry cough

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We have reported a clinically new type of autosomal dominant disorders of motor and sensory neuropathy (Miura et al. J Neurol Sci 2008). The cardinal clinical features of the disease were slow progression, muscular weakness with dominancy in the proximal portion of the lower limbs and in the distal portion of the upper limbs, sensory involvement with dominancy in the lower limbs (glove and stocking type), areflexia, fine postural and action tremors, painful muscle cramps, elevated serum creatine kinase levels, urinary disfunction, and recurrent paroxysmal dry cough. Sural nerve biopsy showed moderate, chronic, mainly axonal neuropathy. To identify the causative nucleotide variant for the disease, we studied the Japanese pedigree with the disease of 19 family members including nine patients in five generations. By the linkage analysis, we mapped the disease locus to 1p13.3-q23 (maximum logarithm-of-odds score = 2.24). The whole-exome sequencing upon five patients and one healthy relative from the pedigree revealed 2,526 patient-specific single nucleotide variants (SNVs). By the filtering processes using the public SNP databases, our linkage analysis, validation by Sanger sequencing, confirming cosegregation, genotyping 520 healthy Japanese individuals, and functional predictions, we identified one SNV located in *IQGAP3* which is known to be associated with neurite outgrowth. Immunohistochemistry show increased expression of IQGAP3 in lymphocytes and sural nerves of a patient compared to those of a control. We conclude that *IQGAP3* is a causative gene for the disease. This is the first record of a disease caused by an IQGAP3 variant.

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P10.14A
Genetics heterogeneity and novel genes in autosomal recessive Hereditary Spastic Paraplegia (AR-HSP)

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Introduction: Hereditary spastic paraplegia (HSPs) is a group of inherited neurodegenerative disorders characterized by progressive spasticity and weakness in lower limbs. The mode of inheritance in HSP can be autosomal-dominant, autosomal-recessive, X-linked, or mitochondrial. There is significant genetic heterogeneity in HSP, with at least 65 genes and 80 loci identified thus far. Whole exome sequencing (WES) has been used for gene discovery in HSP since 2011, resulting in a marked increase in the rate of novel disease-causing genes being identified. Despite the use of WES, genetic analysis has failed in finding of causative genes in ~45%-60% in the autosomal dominant-HSP (AD-HSP) and ~71%-80% in the autosomal recessive-HSP (AR-HSP) groups, indicating that, the majority of HSP-genes especially AR-HSPs have remained unknown.

Materials and Methods: In order to identification of novel HSP-disease causing genes, we investigated the causes of AR-HSP in 12 unrelated Iranian families using WES.

Results: This approach led us to identify the mutations in seven known disease-causing genes including *SPG7* (two cases), *CAPN1, CYP7B1, ENTPD1, GJC2, ERLIN2*, and *SPG11* (two cases) and three novel candidate HSP genes. Functional analyses to evaluate of the biological implication of the novel genes are ongoing.

Conclusions: Here, we could find nine variations in seven known HSP-causing genes in 12 cases (75%) and three novel HSP-genes in the remaining ones using WES method. Identification of novel genes and novel molecular pathways will greatly enhance our understanding of the cellular pathways that are critical for axonal health and our knowledge about pathogenesis of the disease.

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P10.15B
Clinical background of hyper creatine kinase in girls without a family history of genetic muscular disease

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Persistent elevated plasma creatine kinase (CK) raises the suspicion of various conditions such as genetic muscular disease, inflammatory muscular disease, metabolic

myopathy, or hypothyroidism. In girls, carrier status of Duchenne/Becker muscular dystrophy (DMD/BMD) should be taken into consideration so that the diagnostic process might include both technical and ethical aspects. Therefore, no diagnostic system has been established and the clinical background is still unclear. This study aimed to investigate the clinical background of hyper-creatine-kinase-emia girls. Fifteen girls, who were referred to our hospital between April 2014 and August 2018 with persistent elevated plasma CK without a family history of muscular disease, were included. Age and Value of CK at the first visit were 0-12 years old and 740-21,944 IU/L. Eight cases exhibited clinical symptoms (symptomatic group), while seven cases showed no symptoms (asymptomatic group). Genetic muscular disease was diagnosed using muscle biopsy or genetic analysis. In the symptomatic group, diagnoses were DMD/BMD carrier (3 cases), Fukuyama congenital muscular dystrophy (1 case), sarcoglycanopathy (1 case), dermatomyositis (1 case), and undiagnosed (2 cases). One girl presenting with marked elevated CK and developmental delay was diagnosed with balanced translocation with the breakpoint located within the DMD gene and skewing of X-chromosome inactivation. Diagnoses in the asymptomatic group were DMD/BMD carrier (3 cases), calpainopathy (1case), and undiagnosed (3cases). Both groups included patients affected by muscular disease and DMD/BMD carriers, suggesting that both conditions should be considered regardless of presence of symptoms. Building a diagnostic system is necessary based on the clinical background shown in this study.

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P10.16C

Expanding the molecular characterization of the CMT2Z disease associated gene *MORC2*

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Introduction: Mutations in MORC2 lead to an axonal form of Charcot-Marie-Tooth neuropathy (CMT2Z). To date, thirty-one families have been reported, showing that this gene is frequently involved in CMT. However, the phenotypic consequences of MORC2 in patients and its role in neuronal biology remain to be clarified.

Materials and Methods: A gene capture panel was performed to screen undiagnosed patients with CMT. To determine the effect of the most common MORC2 mutations in a neural context, p.S87L and p.R252W, we used a virus-based system in vitro to overexpress MORC2 in sensory neurons. ATPase activity of MORC2 was measured by colorimetric assay. A patients-derived fibroblasts and rat sensory neurons overexpressing clinical mutations were investigated by both transcriptomics and proteomics studies to gain an insight into the pathogenic mechanisms of MORC2.

Results: Here, we report the MORC2 mutations detected in our clinical series, including a review of the reported literature. Moreover, we show that the overexpression of p. S87L mutant in sensory neurons induced significant axonal phenotype. In addition, we observed that p.S87L mutant has an impaired ATPase activity. The data analysis of transcriptomic and proteomic approach in sensory neurons and patients' fibroblasts indicate complex changes affecting transcripts involved in neuronal and axonal functions.

Conclusions: Our data expand the spectrum of clinical mutations in MORC2 and provide an important insight into the pathophysiological role of MORC2 in the nervous system. Funds: AFM-Téléthon, ISCIII (Grant PI15/00187) co-funded with FEDER funds, Prometeo Program from the Generalitat Valenciana and Fundación Ramón Areces.

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P10.17D
Molecular analysis of sarcoglycanopathies in Russian LGMD patients

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Introduction: Sarcoglycanopathies (SGCs, LGMD2C-F) are a subgroup of autosomal-recessive limb-girdle muscular dystrophies (LGMD), caused by mutations in four sarcoglycan genes (*SGCG, SGCA, SGCB, SGCD*). These forms are characterized by childhood onset and rapidly progressive course with loss of ability to walk during a second decade of progression. In general, SGCs could hardly be distinguished from Duchenne/Becker myopathy and other pseudohypertrophic LGMDs due to quite similar clinical symptoms.

Materials and Methods: Targeted massive parallel sequencing was performed in 290 Russian patients with progressive muscle weakness using a custom gene panel covering four sarcoglycan genes.

Results: Genetic analysis revealed homozygous and compound-heterozygous mutations in 16 patients in 3 of 4 sarcoglycan genes. The *SGCA* mutations have been detected in 13 cases (4,5%); the *SGCB* and *SGCG* mutations have been found in 2 (0,7%) and 1 (0,3%) cases respectively. Mutation spectrum of *SGCA* gene was represented by 12 different mutations, 4 of them are recurrent. Previously we also identified 4 patients with *SGCA* mutations in a Duchenne/Becker myopathy cohort. As the result, two pathogenic *SGCA* variants c.229C>T (p.Arg77Cys) and c.271G>A (p.Gly91Ser) were defined as the most common *SGCA* mutations in Russian patients accounting 29,4% and 26,5% of all mutant alleles respectively.

Conclusions: This study revealed that LGMD2D is the most common form of SGCs in Russian LGMD cohort. To sum up, SGCs account 5,5% of all LGMDs in total that make them the third LGMD subgroup following LGMD2A and LGMD2I. However, in contrast to other European populations, SGCs are very uncommon in Russia.

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P10.18A
Identification of *CAPN3* gene novel variations in Iranian LGMD patients

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Introduction: Limb Girdle Muscular Dystrophy type2A (LGMD2A), caused by mutations in *CAPN3* gene, is the most common LGMD form worldwide (30-40% of all LGMD2 cases). LGMD2A is an autosomal recessive disorder highlighted by weakness of the pelvic and shoulder girdle muscles. Recently, *CAPN3* heterozygous mutations have been known to cause dominant LGMD type4 (LGMDD4).

Materials and Methods: We investigated a large cohort of 192 Iranian patients with different grades of muscular dystrophy who referred to Kariminejad-Najmabadi Pathology and Genetics Center during 2012-2018 by exome and targeted next-generation sequencing.

Results: Twenty nine LGMD2A cases were identified, carrying 22 different *CAPN3* gene variants. Approximately 15% of participants possessed variations in *CAPN3* gene; including 10 missense, 5 frameshift, 1 inframe, 2 nonsense and 4 splice site changes; in which 9 variants were novel compromises p.Gly127Glu, p.Ile266_Asp267del, p.Ser265Pro, p.Gly221Cys, p.Ser432*, p.Tyr507Cys, p.Gln545Serfs*50, c.1115+1dup and c.1353A>G at splice donor site. Moreover, 4 variants occurred multiple times in the cohort (p.Ile266_Asp267del, p.Ser432*, p.Arg748Gln and c.1115+1dup). In 3 patients, the second causative variant has not been found which suggests diagnosing of LGMDD4.

Conclusions: Based on findings, it seems that prevalence of LGMD2A is high in Iranian LGMD patients and some mutations are more frequent. Besides, most of the variants have been occurred in Domain III and the catalytic domain (domain II) of the calpain3 protein involved in Ca2+-induced activation of the protein. Defects in these domains lead to impair its autolytic activity. Therefore, designing drugs to target this part of the Calpain3 protein might help to improve the quality of life in patients.

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P10.19B
Multigenic involvement of the semaphorin-plexin pathway in Moebius syndrome by a germline chromothriptic rearrangement

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Moebius syndrome (MBS) is a congenital disorder primarily caused by unilateral or bilateral paralysis of the facial and abducens nerves. Both genetic defects and environmental toxic factors leading to abnormal brainstem development are proposed to be involved in the aetiology of MBS. Recently, mutations in *PLXND1* and *REV3L* were confirmed to cause MBS. Here, we applied next generation mate-pair sequencing to map the breakpoints of a complex chromosomal rearrangement (CCR) 46,XY,t(7;8;11;13) in a previously reported patient with MBS, and confirmed 39 out of 41 breakpoint-junctions by Sanger sequencing. Molecular mapping revealed 41 clustered breakpoints, involving chromosomes 7, 8, 11 and 13, resulting in heavy intra- and interchromosomal rearrangements with typical hallmarks of chromothripsis. Among 12 truncated protein-coding genes, *SEMA3A* and *SEMA3D* are attractive candidates for the MBS features, as they encode class 3 semaphorins, where SEMA3A is known to bind to the MBS-associated PLXND1. Intriguingly, the CCR also truncated *PIK3CG*, which *in silico* interacts with a protein encoded by the other known MBS-gene *REV3L*, and with the SEMA3A/PLXND1 complex via the vascular endothelial growth factor FLT1. In conclusion, the simultaneous truncation of several interactors of the known MBS-genes by a single CCR suggests that the multiple breakpoints in germline chromothripsis may predispose to complex multigenic disorders. **Grants:** Danish Council for Independent Research [4183-00482B]; University of Copenhagen Excellence Programme for Interdisciplinary Research; Lundbeck Foundation [2013-14290]); NIH K99/R00 Pathway to Independence Award; Cancer Prevention Research Institute of Texas Recruitment Award.

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P10.20C
Inactivation of *KLHL24* results in myopathy and cardiomyopathy

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Introduction: Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular disorder, yet the genetic cause remains unknown in up to 50% of the individuals who lack sarcomere gene mutations. We report a novel genetic cause of a cardiomyopathy mimicking HCM in two consanguineous families with a high incidence of sudden cardiac arrest. The age at onset in the patients was in adolescence or in young adulthood.

Material and Methods: Investigations included clinical examination, endomyocardial and skeletal muscle biopsy and genetic analyses by exome sequencing and homozygosity mapping. Functional studies were performed in zebrafish by expression analysis and genetic down-regulation using antisense morpholino oligomers.

Results: Endomyocardial biopsy and skeletal muscle biopsy of individuals from both families demonstrated characteristic alterations including muscle fibers with a "cogwheel" appearance, and desmin accumulation.

Homozygosity mapping and exome sequencing identified a homozygous missense mutation in *KLHL24*, c.917G>A, p. Arg306His in one family and a nonsense mutation, c.1048G>T, p.Glu350* in the second family. *KLHL24* encodes a conserved protein, which is highly expressed in striated muscle. Studies in zebrafish revealed expression of the homolog gene klhl24a in the heart and downregulation by antisense morpholino oligomers resulted in cardiac dysfunction.

Conclusions: We have identified a novel form of cardiomyopathy that mimics HCM with recessive inheritance and onset in adolescence or young adulthood. Genetic, morphological and functional studies demonstrated that inactivation of *KLHL24* is associated with the disease. Several individuals had died of sudden cardiac arrest indicating that lethal arrhythmias may be a common complication.

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P10.21D
Interruptions in the CTG repeat sequence in the DMPK gene in a 40-year-old patient with myotonic dystrophy type I

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Introduction: Myotonic dystrophy type 1 is an autosomal dominant neuromuscular disorder that is caused by the expansion of a CTG triplets in the DMPK gene. We want to present that using a standard TP-PCR method it is possible to skip expansion of CTG triplets due to interruptions present in the CTG repeat sequences.

Materials and Methods: Patient- female (40 years old) symptoms of dystrophia appeared at the age of 30: problems with walking, balance disorders and weakness in the limbs. A test for dynamic mutation in the DMPK gene was performed. Standard TP-PCR method didn't show expansion of CTG repeat sequence. We used a bidirectionally labeled TP-PCR method (Radvansky et al.) in which amplification products are anchored at the 3'end of a CTG repeat expansion rather than the 5' end. The effect of this redesign is that it may prevent the failure in detecting expansion-positive patients carrying repeat interruptions.

Results: TP-PCR reverse primer combinations showed expansion above 50 repeats at the 3'end of the CTG repeat sequence.

Conclusions: Thanks to the combination of both: forward and reverse TP-PCR primers there is a simple and effective method of identifying the presence or absence of expanded CTG repeat alleles in the DMPK gene. It seems necessary to use forward and reverse TP-PCR primers, due to the possibility of occurrence of interruptions observed at both ends of the CTG repeat sequence.

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P10.22A
Validation of a fast, robust, inexpensive, two-tiered neonatal ncreening test algorithm on dried blood spots for spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is one of the leading genetic causes of infant mortality with an incidence of 1:10,000. Recently introduced antisense oligonucleotide treatment improves outcome, in particular when applied presymptomatically. The genetic cause of SMA is in >95% of cases a homozygous deletion of the survival motor neuron (SMN1) 1 gene, which makes low cost detection of SMA cases as part of newborn screening programs feasible. We developed and validated a new SALSA MC002 melting curve assay that meets Dutch legal requirements of not detecting asymptomatic carriers and that detects the absence of the SMN1 exon 7 DNA sequence using crude extracts from dried blood spot (DBS) newborn screening cards. Melting curve analysis shows specific peaks of both the SMN1 and the disease modifying SMN2 homolog, and in case of insufficient amounts of sample DNA, a warning peak. We retrieved 47 DBS samples from children with

genetically confirmed SMA (identified by using the Dutch SMA database) after consent from parents and 375 controls from the national archive of the Dutch National Institute for Public Health and the Environment (RIVM). The assay correctly identified all anonymized SMA and control samples (i.e. sensitivity and specificity of 100%), without the detection of carriers, on 3 most commonly used PCR platforms with melting curve analysis. Concordance with the second-tier 'golden standard' P021 SMA MLPA test was 100%. Using the new P021-B1 version, crude extracts from DBS cards could also be used to determine the SMN2 copy number of SMA patients with high accuracy.

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P10.23B

Improving molecular diagnosis of Charcot-Marie-Tooth disease by targeted next-generation sequencing in a cohort of Lithuanian patients

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Charcot-Marie-Tooth disease (CMT) is the most common hereditary neuropathy with over 100 causative genes identified to date. Next generation sequencing (NGS) approaches represent a powerful tool to identify genetic variants in CMT in contrast to classic time consuming gene by gene approach. However, approximately 50% of patients remain undiagnosed after use of NGS tools. The aim of this study was to identify the molecular diagnosis in a group of patients with CMT using targeted NGS. 38 patients presenting with hereditary neuropathies were tested by targeted NGS for 150 nuclear genes associated with CMT and related neuropathies. In 15 of the 38 patients, we identified the definite molecular diagnosis (39.47%). In total, 6 novel and 8 known pathogenic variants in 12 genes were identified. Autosomal recessive axonal neuropathy with neuromyotonia was the most prevalent type in our cohort (4 patients). Additionally, autosomal recessive inheritance was confirmed in patients harbouring *GDAP1* and *FIG4* variants. Our targeted gene panel allowed us to perform a very rapid and cost-effective screening of genes associated with CMT. We expanded a spectrum of genotypes of CMT and clinical phenotypes of known CMT related genes. This diagnostic rate was achieved by performing accurate neurologic examination prior to enrolment patients in the study and interpreting the NGS data.

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P10.25D

Identification and characterization of disease-causing genes in non-5q-SMA by next-generation sequencing technology: Lessons learned from NeurOmics study

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Introduction: Spinal muscular atrophy (SMA) without deletions/mutations in *SMN1*(non-5q-SMA) is genetically very heterogeneous. Due to strongly overlapping phenotypes of neuromuscular disorders, the differential diagnosis of non-5q-SMAs is challenging.

Materials and Methods: For the last 23 years, we have performed *SMN1* deletion analysis to 3,535 individuals with

suspected SMA. In 1,715 (48.5%) of these individuals, no *SMN1* deletion/mutation was detected. Review of the clinical data with respect to a possible motor neuron disorder led to 114 patients who were available and consented to participate. These 114 patients were analysed by two different gene panels. Of these, 24 unresolved patients and 49 new patients were analysed by WES or/and WGS.

Results: Gene panel provided a diagnosis in 39% (44/114) of the probands. The neuromuscular disease panel with 479 genes outperformed the lower motor neuron disease panel with 62 genes with a 44% to 13% diagnostic yield, respectively. WES/WGS revealed the disease-causing mutation in 38% (28/73) of these patients. Within the NeurOmics project, we published five novel disease-causing genes (*BICD2, CHP1, PIEZO2, VAMP1, ADPRHL2)* and introduced three novel genes (*PRUNE1, MCM3AP, AGTPBP1)* with recently published overlapping phenotypes.

Discussion: NGS is a powerful method for diagnostic and gene identification purposes in non-5q-SMA, and illustrate the wide spectrum of neuromuscular disorders that initially present as motor neuron disease. Gene panels with larger gene representation perform best in clinical conditions with heterogenic genetic background. For phenotypes that are not fulfilling the core clinical criteria for motor neuron disorders, a more comprehensive WES or WGS methods should be used.

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P10.26A
Genotype-driven WES analysis identifies novel compound heterozygous splice-site mutations in*PIEZO2*causing a unique variant of distal arthrogryposis with impaired proprioception & touch

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A family with two siblings (20-year old male & 18-year old female) affected by an undiagnosed Neuromuscular disease was analyzed by WES of a single affected individual to identify the offending gene defect. The clinical presentation includes agenesis of corpus callosum, severe congenital truncal hypotonia, severe scoliosis, short stature, delay in bone age, ataxic gait / jerky walking, triangular face with narrow maxilla affecting speech, congenital clasped thumb and congenital clubfoot (female patient), lateral deviation deformity of foot bilaterally and upper and lower extremity spasticity. Phenotype-driven WES analysis did not identify any known pathogenic or novel predicted damaging variants in known genes. Genotype-driven WES analysis for known pathogenic mutations and novel variants predicted as damaging in MIM genes, identified two novel *PIEZO2* exon 27 splice site mutations (c.4093-1G>C and c.4189+1G>A, both predicted damaging). Recessive *PIEZO2* pathogenic mutations cause Distal Arthrogryposis with Impaired Proprioception & Touch (DAIPT) with clinical presentation that overlaps that of the patients under investigation. Mutation-to-disease co-segregation in the nuclear family by Sanger sequencing, showed the variants in "trans" configuration as both parents were carriers of a different mutation and the affected siblings compound heterozygotes. Phenotype-driven WES analysis failed to identify the offending gene most likely due to clinical heterogeneity. Agenesis of corpus callosum has not been associated with DAIPT. Impaired proprioception was not properly diagnosed, and arthrogryposis was erroneously referred as "spasticity". Genotype-driven WES analysis is an invaluable tool that should be utilized rather than phenotype-driving analysis to accommodate for clinical heterogeneity and variability in clinical presentation.

E. Darmani: A. Employment (full or part-time); Significant; ANALYSI IATRIKI SA. **K. Kydonopoulou:** A. Employment (full or part-time); Significant; ANALYSI IATRIKI SA. **E. Papadopoulou:** A. Employment (full or part-time); Significant; ANALYSI IATRIKI SA. **S. Gerou:** A. Employment (full or part-time); Significant; ANALYSI IATRIKI SA. **M. Kambouris:** None.

P10.27B
Distinguishing Highly Similar *SMN1/2* Genes and Identifying Novel SMN Transcripts by Targeted Capture of PacBio Single-molecule Long-read Sequencing

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Introduction: Spinal muscular atrophy (SMA) is autosomal recessively inherited disease with a high carrier frequency caused by biallelic mutations in *SMN1* gene. As high homology between *SMN1* and *SMN2*, it would be difficult

to distinguish the mutations in *SMN1* from *SMN2*. PacBio single molecule real time (SMRT) sequencing techniques can aids the identification of transcript variants due to the longer read lengths

Materials and Methods: Twenty-one study subjects were selected from the diagnostic laboratory. Multiplex PCR with four pairs of specific-primers to enrich SMN transcripts. Enriched products sequenced by long-read sequencing approach using SMRT. An 20bp anchor sequences were used to distinguish *SMN1* and *SMN2*. Marked with *SMN1/SMN2* reads utilized GATK for variation calling.

Results: For the majority of subjects in this study, *SMN1* transcripts full-length SMN mRNA (FL-SMN) and *SMN2* transcripts lacking exon 7 mRNA (SMNΔ7) as reported. Nine in ten subjects with subtle mutation are detected by long-read sequencing. Detected mutation of M0 are existing in SMNΔ7, all the other detected mutation are existing in FL-SMN. There are ten novel transcripts detected. Six of ten are successfully amplified by PCR.

Conclusions: We have developed a novel method to distinguish highly similar *SMN1* and *SMN2* genes and identify affected mutations in *SMN1* or *SMN2* by long-read SMRT sequencing. Meanwhile, the method can comprehensively illustrate SMN transcripts existed.

M. Dai: None. **Y. Xu:** None. **X. Ji:** None.

P10.28C
Facioscapulohumeral Muscular Dystrophy (FSHD): insights into the genetic characterization of *SMCHD1*

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Introduction: *SMCHD1* (18p11.32) is a gene coding for a chromatin remodeller involved in the epigenetic silencing of different targets, including genes located in *D4Z4* locus (4q35). *SMCHD1* loss of function mutations can hamper its function, leading to the aberrant expression of *D4Z4* genes associated with FSHD. This study aimed to characterize a group of patients with a clinical suspect of FSHD, by molecular analysis of *SMCHD1* sequence.

Materials and Methods: 58 patients with a clinical suspect of FSHD were enrolled and were analyzed by NGS methodology and direct sequencing. The detected variants were investigated by bioinformatics tools in order to predict their impact on the protein and evaluate the potential pathogenic effect on FSHD.

Results: Molecular analysis of *SMCHD1* sequence revealed the presence of exonic, intronic and 3'UTR variants. Among them, five novel variants (c.183_184insGT; c.1131+2delTAAG; c.2130insC; c.3469G>T; c.5150_5151delAA) were predicted to be pathogenic for FSHD, causing a substantial alteration of protein structure and function. In addition, four variants (c.7394A>C; c.7597G>A; c.*1397A>G; c.*1889G>C) in 3'UTR were predicted to affect the binding of different miRNAs.

Conclusions: Molecular analysis of *SMCHD1* allowed to detect novel potential pathogenic variants in patients with a clinical suspect of FSHD. In particular, those variants may modify the structure and function of SMCHD1 protein, leading thereby to the disruption of its physiological activity and, ultimately, contributing to FSHD etiopathogenesis. Moreover, the 3'UTR variants unveiled a possible impact of miRNA-dependent regulation on FSHD-related pathways.

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P10.29D
Screening multiple populations reveals large differences in the prevalence of a truncated SMN gene lacking exon 7 and 8

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Introduction: Spinal muscular atrophy (SMA) is one of the most frequent genetic disorders in Caucasians, with an incidence of approximately 1:10.000 in newborns. SMA is an autosomal recessive disorder with a population dependent carrier frequency ranging from 1:35 in Caucasians to 1:91 in African Americans. In the majority of populations, most individuals have two copies of both *SMN1* and the almost identical *SMN2* gene. Previous studies have identified the presence of *SMN1* or *SMN2* gene copies lacking the last two exons, exons 7 and 8, in 8% and 23% of healthy Swedish and Spanish individuals respectively. No

information on the presence of these truncated gene copies in other populations has been reported.

Methods: A new version of the SALSA MLPA P021 SMA probemix was tested on 1255 samples from the 1000 genomes / HapMap projects from 15 different populations. The probemix covers all *SMN1* and *SMN2* exons by at least one probe and contains four *SMN1* or *SMN2* specific probes.

Results: Large differences in the frequency of a truncated SMN gene were observed between all the different populations tested, ranging from 0 to 20%. Frequencies in the different populations were as follows: 8-20% in European populations, 7-11% in American populations, and 0-1% in both the Asian and African populations.

Conclusion: The improved P021 MLPA Probemix makes the identification of truncated SMN genes straightforward. These truncated SMN genes are found frequently in several populations while they are relatively non-existent in other populations with their clinical significance still unknown.

R. Vijzelaar: A. Employment (full or part-time); Significant; MRC-Holland. **R. Snetselaar:** A. Employment (full or part-time); Significant; MRC-Holland. **M. Clausen:** A. Employment (full or part-time); Significant; MRC-Holland. **A. Mason:** A. Employment (full or part-time); Significant; MRC-Holland. **M. Rinsma:** A. Employment (full or part-time); Significant; MRC-Holland. **M. Zegers:** A. Employment (full or part-time); Significant; MRC-Holland. **N. Molleman:** A. Employment (full or part-time); Significant; MRC-Holland. **R. Boschloo:** A. Employment (full or part-time); Significant; MRC-Holland. **R. Yilmaz:** A. Employment (full or part-time); Significant; MRC-Holland. **R. Kuilboer:** A. Employment (full or part-time); Significant; MRC-Holland. **S. Sulchan:** A. Employment (full or part-time); Significant; MRC-Holland. **J. Schouten:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; MRC-Holland.

P10.30A
SARS2 modifies spastic paraplegia type 4 age at onset

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Introduction: Hereditary Spastic Paraplegias (HSPs) are rare neurological disorders caused by the progressive distal degeneration of the corticospinal tracts. Mutations in *SPAST*, encoding Spastin, are the most frequent cause of HSP. Recently, through the analysis of 842 *SPAST* mutated patients, we showed that mutation nature accounts for some of the age at onset variability and that sex influences the disorder penetrance. To identify additional age at onset modifiers, a GWAS analysis was performed on 134 *SPAST* truncating mutations carriers.

Materials and Methods: Patients were genotyped using Illumina InfiniumOmni2.5Exome-8 kit. Single variant test was performed using a logistic mixed model for binary traits available with GMMAT R package, adjusted for sex and patients' relatedness. Patients with very discordant age at onset were compared (≤15 years versus ≥45 years).

Results: A group of SNPs in linkage disequilibrium (r^2>0.8) reached a p-value suggestive for association (top p-value = 10e-6). The 7 suggestive SNPs are located on chromosome 19, in the 3'UTR region of *SARS2* gene and are described as eQTLs of this gene in the GTEx database. Among the genotyped patients, a significantly lower age at onset (p<0.0001, Mann-Whitney test) was associated with the minor allele at all SNPs, as well as a tendency to a decreased disorder severity.

Conclusions: Pathogenic mutations affecting *SARS2*, encoding a mitochondrial seryl-tRNA synthetase, have already been linked to progressive spastic paraparesis onset, suggesting it as a good candidate. iPSCs-derived neurons and *Drosophila* model are being used to further confirm its role as *SPAST*-HSP age of onset modifier.

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P10.31B
Development of motor function in late vs early treatment with nusinersen for SMA type 1 and 2 patients

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Introduction: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in the SMN1-gene (5q 12.2-13.3) generating atrophy of anterior horn cells in the spinal cord and brainstem leading to muscle weakness. The antisense oligonucleotide nusinersen was recently approved in Sweden for treatment of SMA in the public health care system. In this study we compare the development in motor function in patients who received treatment within three

months of symptom onset for SMA type 1, and within twelve months for SMA type 2, to the patients receiving treatment later in the course of disease.

Materials and Methods: All patients diagnosed with SMA type 1 or 2 treated with nusinersen for at least twelve months at the Department of Pediatrics, Queen Silvia Children's Hospital, Gothenburg, Sweden are included in the study. The motor function is assessed by standardized motor function scales; CHOP-INTEND/HFMSE, developed and validated for SMA patients.

Results: Data regarding age of onset, age at start of treatment, genetic constitution and development of motor function will be presented.

Conclusion: Previous studies have indicated that treatment efficacy for motor function is greater in patients receiving treatment close to onset of symptoms or even in presymptomatic patients compared to patients receiving the treatment later in the course of disease. This highlights the importance of early diagnosis and raises the question of inclusion of SMA in newborn screening programmes.

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P10.32C

Methylation level of genes on various stages of differentiation of induced pluripotent stem cells, generated from patients with spinal muscular atrophy, into motoneurons

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Spinal muscular atrophy (SMA) is a severe neuromuscular disorder caused by mutations in the *SMN1* gene. SMA is subdivided into four clinical groups based on age of onset and achieved motor milestones. *SMN2* gene copy number is a key modifier of the disease, though involvement of additional factors such as DNA methylation is also examined. Previous studies have demonstrated correlation between methylation profiles of several genes and SMA severity. Current study is devoted to investigation of methylation level of genes in SMA patients' derived cells on sequential stages of differentiation of induced pluripotent stem cells (iPS) into motor neurons. The level of methylation of *SMN2* gene as well as genes playing role in neural cells (*OLIG2*, *ISL1*, *SLC23A2*, *DYNC1H1*) and pluripotency-regulating genes (*Oct4*, *SALL4*) has been determined by means of methylation-sensitive high-resolution melting. Significant differences have been found in the methylation level of the regulatory regions of *OCT4*, *SALL4* and *SMN* genes between patients with SMA type I, type II and a healthy individual. A decrease in methylation level of the promoter region of *OLIG2* gene in SMA type I compared to SMA type II, as well as of 41 exons of the *DYNC1H1* gene in SMA type I compared to a healthy individual was also found. The results observed in this study may elucidate new pathways involved in SMA progression and may contribute to better understanding of the particularities of disease pathogenesis. This work is supported by Russian Foundation for Basic Research grant 18-315-00258 mol_a.

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P10.33D

Mutation Spectrum of the Survival of Motor Neuron 1 in Iranian Population

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Spinal muscular atrophy (SMA) is a lethal disorder characterized by loss of motor neurons, muscle weakness, hypotonia and muscle atrophy. SMA is highly heterogeneous with various genes with different heritable patterns causing the disease. In the typical form, patients have defective *SMN1* genes. To date, tens of genes have been identified for Atypical SMA. In this study, 144 families

suspected for SMA were included. Patients were first examined for typical SMA by MLPA and assessment of STR markers for *SMN1*. Next, patients who did not have mutations in the *SMN1* gene were examined using STR markers for the *DNAJB2*, *IGHMBP2*, *SIGMAR1* and *PLEKHG5* genes. Subsequently, autozygosity mapping followed by sequencing were performed for cases with homozygous haplotypes. Finally, Whole Exome sequencing (WES) was done for the remaining cases who did not show association with any of the studied genes. The pathogenicity of the newly identified mutations was examined using various softwares and ACMG guideline.*SMN1* deletions/mutations were identified in 134 families. Of the remaining 10 families, three families showed mutations in *DNAJB2*, *SIGMAR1* and *PLEKHG5* genes. In six families tested by WES, four families had pathogenic variants in *TNNT1*, *TPM3* and *TTN* genes respectively. MLPA and assessment of STR markers can be helpful in both typical and atypical SMA cases. Moreover, linkage analysis and WES are suggested as effective strategies for identification of mutations in patients with atypical SMA.

M. Taheri: None. **A. Sayad:** None.

P10.34A

Accurately resolving copy number variation in highly homologous SMN1 and SMN2 genes using next-generation sequencing and rhPCR

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Introduction: Spinal muscular atrophy (SMA) is a neuromuscular disorder characterized by progressive degeneration of spinal cord motor neurons. In most cases, the disease is caused by the homozygous absence of the *SMN1* gene. Copy number of the highly homologous *SMN2* gene can also modify the disease severity. To address the clinical importance of accurate *SMN1* and *SMN2* copy number analysis, we developed a custom bioinformatic analysis based on next-generation sequencing (NGS) data combined with a novel RNase H2-dependent PCR (rhPCR) for confirmation analysis.

Materials and Methods: The bioinformatic method utilizes sequence reads at four loci differing between the genes. The rhPCR assays target two of the loci with RNA-modified primers that require activation by RNase H2 enzyme improving assay specificity and sensitivity compared to standard methods. We validated the methods using 27 reference samples with known *SMN1*/*SMN2* copy numbers and evaluated the clinical performance by analyzing 89 patient samples.

Results: Both methods showed 100% sensitivity and specificity to detect *SMN1* copy numbers 0-2 or higher and *SMN2* copy numbers 0-3 or higher. In clinical samples, the obtained *SMN1* and *SMN2* copy numbers showed 100% concordance between the methods. Additionally, we applied the bioinformatic analysis to 2196 de-identified patient samples not referred for the indication of SMA. Heterozygous *SMN1* deletions were observed in 2.6% of the samples, which agrees with previously reported frequencies.

Conclusions: We established an accurate and high-throughput approach to test for *SMN1* and *SMN2* copy numbers enabling diagnostics of SMA and application of novel therapeutic strategies.

H. Junnila: A. Employment (full or part-time); Significant; Blueprint Genetics. **M. Valori:** A. Employment (full or part-time); Significant; Blueprint Genetics. **A. Korppoo:** A. Employment (full or part-time); Significant; Blueprint Genetics. **L. Koskinen:** A. Employment (full or part-time); Significant; Blueprint Genetics. **E. Salminen:** A. Employment (full or part-time); Significant; Blueprint Genetics. **K. Gall:** A. Employment (full or part-time); Significant; Blueprint Genetics. **T. Alastalo:** A. Employment (full or part-time); Significant; Blueprint Genetics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Blueprint Genetics. **S. Myllykangas:** A. Employment (full or part-time); Significant; Blueprint Genetics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Blueprint Genetics. **J. Koskenvuo:** A. Employment (full or part-time); Significant; Blueprint Genetics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Blueprint Genetics. **P. Salmenperä:** A. Employment (full or part-time); Significant; Blueprint Genetics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Blueprint Genetics. **J. Sistonen:** A. Employment (full or part-time); Significant; Blueprint Genetics.

P10.35B

Accurate single-tube quantification of *SMN1* and *SMN2* copy numbers using a rapid and streamlined PCR/CE assay evaluated at two different laboratories

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Introduction: Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disease and a leading genetic cause of infant mortality. SMA is commonly caused by homozygous exon 7 deletions in the survival motor neuron 1 gene (*SMN1*). The severity of SMA is modulated by the copy number of the paralogous *SMN2* gene. Here, we report the two-site evaluation of a single-tube assay that quantifies *SMN1* and *SMN2* copy number.

Materials and Methods: A prototype *SMN1/2* single-tube PCR was developed from AmplideX® PCR/CE *SMN1* reagents (Asuragen). Amplicons generated from whole blood genomic DNA were resolved by capillary electrophoresis (CE) on a 3130 Genetic Analyzer (Thermo Fisher Scientific). Gene copy numbers were calculated from peak area ratios that were normalized to a plate calibrator and binned as 0, 1, 2, 3, or ≥4 copies. Two laboratories (one in Portugal and one in the US) evaluated 60 residual clinical samples with reference genotypes.

Results: Normalized *SMN1* and *SMN2* copy numbers demonstrated operator-to-operator and site-to-site agreement (R^2>0.98). Binned copy-number calls for *SMN1* spanned 0 to ≥4 copies and were concordant with reference results for both laboratories. *SMN2* copy numbers ranged from 0 to ≥4 copies and were concordant for 59/60 (98.3%) samples.

Conclusions: A simple, high-throughput *SMN1/2* PCR/CE assay was successfully evaluated using 60 samples, including 15 SMA and 13 carrier samples. This method offers a simple workflow and fast turnaround time (< 4hrs from sample-to-answer) with potential as an accurate and reliable alternative to existing methods.

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P10.36C

Analysis of DNA tandem repeats in ALS from Whole Genome Sequencing: Role of FRA10Ac1 gene repeat expansion in ALS

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The *C9ORF72* gene repeat expansion is the most frequent cause of ALS. Long repeats alleles in ATXN-1, ATXN-2, and NIPA1 genes are associated to ALS susceptibility. Tandem Repeat Polymorphisms (TRPs) are good candidates for missing hereditability in ALS (40%), although they were never systematically analyzed as they represent a remarkable challenge to NGS. The aim of this study is to perform a systematic analysis of TRPs in ALS by combining NGS and novel bioinformatics tools. We performed our analysis from whole genome sequencing data (WGS) of 70 ALS cases. TRPs were evaluated by means of a software developed within our consortium to detect tandem repeat expansion. Validation of expanded loci was conducted by Repeat primer PCR We identified an ALS patient with a CGG expansion in 5'UTR of *FRA10AC1* gene. To explore the possible role of this CGG expansion in ALS we screened a cohort of 337 ALS and 285 controls and we found 3 expanded patients (0.9%) and no control. We failed to replicated this result in a second cohort of 426 ALS patients and 733 controls (1 ALS (0.2%) and 5 controls (0.7%)) Overall, we observed the CGG expansion in 4/763 (0.5%) ALS patients and 5/1018 controls (0.5%). FRA10AC1 gene expression was not silenced by the expansion. The software we developed can detect repeat expansions from WGS data, although *FRA10AC1* CGG expansion seems not to be involved in ALS pathogenesis. Conversely to what previously reported, large CGG expansion at this locus do not decrease gene expression

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P10.37D

Clinical diagnosis of spinocerebellar ataxias (SCAs) and hereditary spastic paraplegia (HSPs) in Portugal using exome sequencing

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SCAs and HSPs are rare genetic neurological diseases with a prevalence of 12.9/100,000 in Portugal. Clinical symptoms often distinguish SCAs from HSPs; however, complex forms combine these and other neurological symptoms, in a wide phenotypic diversity (age-at-onset, severity and progression rate). In this study, we provide an estimate of the diagnostic yield of SCAs and HSPs at our laboratory. We performed whole-exome sequencing (WES) and applied virtual gene panels on 333 patients with SCAs and HSPs, and analysed SNVs, using an in house pipeline, and CNVs, using Golden Helix's VarSeq software. We tested 161 SCA patients using a WES-based virtual panel (145 genes), where we achieved a molecular diagnosis for 17 patients (18 previously described and 13 novel disease-causing variants); in another 72 patients, we reported a VUS, according to the ACMG classification. In 172 patients with HSP (118 genes in the virtual panel), we established a molecular diagnosis in 27 cases (35 described and 8 novel disease-causing variants), while in 50 probands we reported a VUS. We obtained a diagnostic yield of 10.6% for SCAs and 15.7% for HSPs; VUS reported represented 44.7% of SCA and 29.1% of HSP cases. In undiagnosed patients, family studies, correlation with phenotype, CNV analysis in addition to flexible virtual gene panels (e.g., including newly discovered genes or using a large neurological disorder multigene panel) may increase the diagnostic yield, in order to provide a better disease management and improve genetic counselling for affected patients and their families.

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P11
Multiple malformation/anomalies syndromes

P11.01A
Sex-specific transcriptome analysis highlights immune and neuronal dysfunction in the developing brain cortex of 16p11.2 deletion mouse models

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Rearrangements of the proximal 16p11.2 BP4-BP5 region are associated with mirroring anthropometric traits and neurodevelopmental disorders. To assess the molecular impact of 16p11.2 dosage and investigate possible sex interaction(s), we profiled the transcriptomes of brain cortices of wild-type, 16p11.2 deletion and 16p11.2 duplication male and female mouse models across embryonic (E11.5), newborn (P0) and adult (P77) stages. Whereas we corroborate previous results by identifying expression changes of transcripts associated with primary cilium and metabolism, we also uncovered significant perturbation of immune-related genes that encode proteins influencing neuronal development and synaptic function (P=6.44E-05) in 16p11.2$^{Del/+}$ mice. This signal is already present in embryos and newborns, and becomes especially exacerbated in adult females with 6203 differentially expressed genes compared to 146 in males, suggesting that hormonal changes could be involved. This massive transcriptome modification could be a compensation mechanism of 16p11.2$^{Del/+}$ females, consistent with the milder phenotypes of human female deletion carriers. Unfortunately, the vast majority of 16p11.2 animal models have not been studied in a sex-specific manner. We also assessed the transcriptome of 16p11.2$^{Dup/+}$ mice, and in agreement with the hypothesis that aneuploidy of the genes of the rearranged interval is more detrimental than their overexpression, we found, at all developmental stages, greater transcriptional alterations in 16p11.2$^{Del/+}$ rather than 16p11.2$^{Dup/+}$ mice. Our data reveal time- and sex-specific transcriptional changes in the brain cortex of 16p11.2$^{Del/+}$ mice, and shed light on possible intimate connections between the immune and nervous systems and its influence on neurologic manifestations in carriers of 16p11.2 rearrangements.

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P11.02B
Duplication 17q24.3q25.1, inserted on 17p13, encompassing *SOX9:* the impact on the phenotype

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Intrachromosomal insertions are uncommon rearrangements. The cytogenetic recognition of these structurally rearranged chromosomes can be difficult to ascertain. In last years the application of array-CGH in the investigation of patients with intellectual disability and congenital malformations increased substantially the detection of cryptic chromosomal imbalances. However, the rearrangement underlying the imbalance could be missed, when we only perform molecular techniques.

We report a 10 year-old girl presenting intellectual disability, attention deficit hyperactivity disorder, short stature, prenatal microcephaly, aplasia cutis congenita, bulbous bifid nose, bilateral congenital trigger thumb, shortening of the fourth metacarpal of the right hand, pectus excavatum, long first toe, and mild hypertrichosis.

High resolution cytogenetic analysis revealed a cryptic alteration: 46,XX,add(17)(p13.1). To characterize the imbalance 180K oligonucleotide array-CGH was performed, revealing a 2.4 Mb duplication: arr[hg19] 17q24.3q25.1(68,620,187-71,083,594)x3 encompassing 10 genes. Molecular cytogenetics showed an intrachromosomal insertion on 17p. Parents were not available to study. The most relevant gene involved in the imbalance is *SOX9* (MIM*608160) and encodes a transcription factor essential for both sex and skeletal development. Duplications of noncoding elements 5-prime of *SOX9* were associated with abnormal digit and nail development. Investigations are being addressed, by chromosome conformation capture (HiC) in patient cell lines in combination with whole-genome sequencing, in order to do a better interpretation of the pathogenic mechanisms underlying the chromosomal rearrangement.

This report emphasis the contribution of high resolution cytogenetics, molecular cytogenetics and array-CGH to a better understanding of the rearrangement involved in the imbalance, providing a better interpretation and correlation to the phenotype.

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P11.03C
Understanding microcephaly/macrocephaly mechanisms in the new 19p13.3 microduplication/ microdeletion syndrome

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Introduction: A small but growing body of scientific literature is emerging about clinical findings in patients with 19p13.3 rearrangements. Most reported individuals have deletions, and only eleven patients with clinical findings attributed to 19p13.3 duplications have been reported. Interestingly, 19p13.3 microdeletions are described in patients with syndromic intellectual disability and macrocephaly, and 19p13.3 microduplications in patients with syndromic intellectual disability and microcephaly. Moreover, patients with 19p13.3 microduplication share common developmental features, suggesting the existence of a new microduplication syndrome.

Methods: To characterize these new syndromes, we formed a French collaborative study, and included 37 new patients with 19p13.3 rearrangements. We performed phenotype and genotype analysis, including genotype-

phenotype correlations by critical region delineation. We screened this critical region with experimental findings in murine model by using the data-mining software Manteia, to purpose candidate genes never associated with human phenotype before.

Results: We report the largest cohort of patients with 19p13.3 rearrangements, and describe a new 377 Kb critical region associated with syndromic intellectual disability and micro/macrocephaly. 19p13.3 rearrangements are associated with specific recurrent clinical findings: mild to moderate intellectual disability, pre and post-natal growth delay, micro or macrocephaly, osteoarticular anomalies including precocious osteoporosis in children, congenital severe hip dysplasia and scoliosis, congenital heart defect, genitourinary findings and immunodeficiency. Breakpoint analysis and supporting murine model allowed us to purpose candidate genes associated with micro/macrocephaly.

Conclusions: 19p13.3 rearrangements are associated with specific clinical findings, including intellectual disability and micro/macrocephaly. We delineate genotype-phenotype correlations and purposed candidate genes never associated with human phenotype before.

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P11.04D

22q11.2 Deletion syndrome and coexisting conditions - an important prognostic, management, and genetic counseling consideration

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Background: 22q11.2DS is the most frequent CNV, affecting ~1/1000 fetuses and ~1/2000–4000 children, resulting in recognizable but variable findings across multiple organ systems. Patients with atypical features should prompt consideration of coexisting diagnoses due to the possibility of additional genome-wide mutations/CNVs which may be inherited, as well as, mutations/CNVs on the other chromosome 22q11.2 allele resulting in an autosomal recessive condition. Both occurrences compound symptoms impacting management and genetic counseling.

Methods: Records on 1422 patients with laboratory confirmed 22q11.2DS followed at Children's Hospital of Philadelphia were reviewed to identify a dual diagnosis, including a subset with atypical features, e.g. craniosynostosis, whose samples underwent NGS of the intact 22q11.2 allele in Leuven/Warsaw.

Results: 24 patients had a coexisting diagnosis (1.7% of our cohort) including 8 patients with conditions unrelated to 22q11.2DS (SCID, Trisomy 8 mosaic, *CHD7* mutation, cystic fibrosis, maternally inherited 17q12 deletion, G6PD deficiency, von Willebrand disease, 1q21.1 deletion) and 16 patients with 22q11.2DS and a CNV/mutation on the remaining allele resulting in an autosomal recessive condition. The latter group included mutations/CNVs in *GP1BB*, *CDC45* (4), *LZTR1* (2), *SNAP29* (4), and *TANGO2* (5) explaining their atypical features, unfortunately including sudden death in a 5-year-old child with post mortem identification of *TANGO2* Related Disease.

Conclusions: These findings support considering additional laboratory testing in this population to ensure appropriate personalized care, as formulating medical management decisions hinges on establishing the correct diagnoses in their entirety, especially given that these findings are medically actionable, potentially altering long-term outcome and recurrence risk counseling.

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P11.05A

SNVs and CNVs influencing phenotype variability in the patients with 22q11.2 Deletion Syndrome detected from whole exome sequencing data

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The 22q11.2DS has an estimated frequency of 1 in 3000 live births. There is still very poor understanding of how heterozygous microdeletion of approximately 50 genes can lead to such diverse expressivity of the clinical features in patients carrying the same deletions. Two aspects seem to have an impact on phenotype variability; coexistence of the second condition and modifiers of typical clinical features. In this project we focused on three of the potential mechanisms underlying the first aspect: pathogenic variants within the remaining 22q11.2 region, SNVs in genes outside of the deletion and additional CNVs.

For 85 22q11DS carriers deep phenotype was performed based on the form with over 230 clinical features. Subsequently for all patients whole exome sequencing (WES) was carried out. Data was analysed using standard protocol, additionally two algorithms were implemented to identify CNVs in genome (CoNIFER and HMZdelfinder).

WES analysis revealed: 9 pathogenic SNVs and 54 potentially pathogenic variants in known disease related genes correlating with patients' phenotypes. Moreover we found 149 rare variants of unknown significance, including 2 in 22q11.2 region. All variants were classified as deleterious by at least 3 of 4 applied function prediction scores and located in genes with increased expression in brain, nervous system, immune system or heart. CoNIFER algorithm identified 12 rare CNVs and HMZdelfinder 14 deletions.

Conclusions: The, second hit" – pathogenic/potentially pathogenic SNV or CNV may act as a phenotype modifier, changes patient's prognosis and should be considered in genetic counselling.

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P11.06B
Morbidity is increased in 47,XYY syndrome: a nationwide registry study

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Background: The 47,XYY syndrome is common as being diagnosed among 18 per 100,000 newborn males. However, knowledge of the long-term health outcomes of this condition is sparse.

Aim: To describe morbidity in a national 47,XYY cohort using complete registry data on hospital admissions and prescribed medication.

Setting: A uniform public health care system.

Participants: A total of 251 males with 47,XYY (n=205), 46,XY/47,XYY (n=28) or compatible karyotypes (n=18) diagnosed during 1965-2014 and a randomly selected age-matched control cohort of 25,100 males from the general population.

Results: Hospital admission owing to any diagnosis was significantly increased among 47,XYY compared to controls (HR=1.8, CI: 1.6-2.4). Among a total of 18 diagnostic groups the risk of admission was increased in all but three groups. The highest HRs were observed for congenital malformations (HR=6.1, CI: 4.8-7.6); psychiatric diseases (HR=5.7, CI: 4.5-7.1); endocrine and metabolic disorders (HR=3.2, CI: 2.4-4.4); neurologic diseases (HR=3.0, CI: 2.2-4.0); and urogenital system disorders (HR=3.0, CI: 2.4-3.7). Overall, 47,XYY had an increased risk of receiving medicinal prescriptions compared to controls (HR=1.3, CI:1.1-1.5), and it was significantly increased in 11 out of 14 medicinal groups. The highest HRs were observed for medication related to the blood (HR=2.5, CI:1.8-3.5); the nervous system (HR=2.2, CI:1.9-2.7); as well as for urogenital system disorders and sex hormones (HR=2.7, CI: 2.0-3.7).

Conclusions: The 47,XYY syndrome is associated with an increased morbidity as interpreted from data of hospital admissions and medicinal prescriptions. Whether these data extend to the approximately 80% of 47,XYY suffering from non-diagnosis remains unknown.

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P11.08D
Characterization of a CRISPR-Cas9 cellular model to Alström syndrome

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Alström syndrome is a rare disease with a prevalence of 1/1,000,000 per inhabitant. ALMS1 gene has been implicated in this disease, with more than 290 causal mutations, mostly of them located in exons 8, 10 and 16. The majority of these mutations leads to a change of the reading frame that concludes in a premature stop codon, resulting in a truncated protein. The modelling of this disease through KO models

becomes in this context an interesting tool to understand the cellular processes that take place in this disease. We have generated a Knock-out (KO) model in HeLa cell line. We use the CRISPR/Cas9 method with dual system with Homology Direct Repair (HDR). After isolation of homozygous clones, we validated ALMS1 expression by qPCR and Sanger sequencing of the recombinant amplicon comprised between Exon 1 and 3. Then we evaluate mitochondrial activity, proliferation and apoptosis resistance. We obtained a total inhibition in the expression of the ALMS1 gene. We are performing the characterization but we expected apoptosis resistance, cell cycle elongation and inhibition of mitochondrial activity like other authors report in analysis of patient´s fibroblasts. The generation of cellular models using CRIPR/Cas9 for the Alström syndrome is considered as a simple and easy to implement tool that would broaden the knowledge of the molecular basis of this disease.

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P11.09A

Beyond the first clinical diagnosis: a new interesting case of KBG syndrome

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A 48 years old man acceded in emergency unit for addominal pain and vomit. He presented polyuria, polydipsia for 2 months and important weight loss. Hematic exam showed high glycemia, high value of HB1Ac and negative specific insulin autoantibodies suspecting diabetic ketoacidosis. The insulin treatment improved symptoms and glicemia. The genetic evaluation revealed a more complex phenotype included hyposomia, growth delay, mild intellectual disability, monosomy of left kidney, fusion of cervical vertebrates, brachydactyly, Arnold-Chiari syndrome and chronic lymphocytic leucemia. The WES analysis confirmed the multiple aetiopatology of the clinical picture identifying mutations in different causative genes. The likely pathogenetic missense mutations in *CAPN10*, *SLC2A2* and *GCKR* explained only diabetes mellitus non-insulin dependent. The kidney anomaly correlated to *CHD1L* mutation, candidate gene for congenital anomalies of the kidney and urinary tract (CAKUT). An essential in-depth study of WES identified a new *ANKRD11* mutation, responsible of KBG syndrome. This is a rare autosomal dominant condition characterized by short stature, neurological involvement, skeletal anomalies and macrodontia. *ANKRD11* encodes for a member of family of Ankyrin repeat domain-containing cofactors that are inhibitors of ligand-dependent transcriptional activation. Moreover, it is known as a tumor protein p53-interacting protein. ANKRD11 enhances TP53-dependent transcriptional activation promoting its tumor-suppressive effects. Retrospectively analysis of literature (about hundred cases) highlighted the presence of only two cases with malignancy, testicular rhabdoid tumor and acute myeloid leukemia. Although we cannot exclude that tumor observed in KBG patients developed coincidentally, the role of ANKRD11 and our new case suggest a general cancer surveillance.

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P11.10B

Fruits of Genomic Match-making: De Novo Variants in *PRR12* are Associated with a Spectrum of Eye and Neurodevelopmental Anomalies

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Rare disease research has made great strides in the last decade; first with unbiased genome-wide sequencing methods, then with global data-sharing efforts. Genomic match-making has accelerated the path from candidate gene identification to establishing causality. Using exome sequencing, we identified a de novo frameshift variant in the *PRR12* gene in a 2-year-old girl with anophthalmia and developmental delay. *PRR12* is highly expressed in the brain and the visual system. It has a high missense constraint score (Z=3.37) and no loss of function variants listed in Gnomad (pLI=1.00). De novo truncating variants in *PRR12* were recently reported in three individuals with developmental delay and iris abnormalities. Through GeneMatcher, we were connected to 11 additional patients from around the globe, bringing the total number of individuals with de novo variants in *PRR12* to 15. There were no recurrent variants; all but one, were truncating; and none were listed in Gnomad. A variety of eye abnormalities were observed in 10/15 individuals; including myopia, stellate iris, coloboma, Rieger's anomaly, microphthalmia, cryptophthalmos and anophthalmia. Developmental delay was noted in 13/15 and microcephaly in 5/15. The overlapping clinical findings, especially the ophthalmological features, support an association between haploinsufficiency of *PRR12* and a distinct neurodevelopmental disorder. Features are variable and functional data is needed to confirm pathogenicity. We plan to use zebrafish and mouse models to replicate the eye and growth phenotypes and further investigate the pathogenicity of different *PRR12* variants. This cohort once again demonstrates the utility of global data sharing efforts in rare disease research.

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P11.11C

Skeletal defects and defective osteoclast and osteoblast function in Aymé-Gripp syndrome

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Ayme-Gripp syndrome (AYGRPS) is the eponym of a recognizable condition caused by a restricted spectrum of dominantly acting missense mutations affecting the basic leucine zipper (bZIP)-containing transcription factor, *MAF*. Major clinical features include congenital cataracts, sensorineural hearing loss, intellectual disability, seizures, reduced growth, and a distinctive flat facial appearance. Skeletal abnormalities have also been noted in affected individuals; even thought, these features have not been assessed systematically due to the small number of subjects. AYGRPS-causing *MAF* mutations cluster in the GSK3 phosphorylation motif within the N-terminal transactivation domain of the protein, and impair proper phosphorylation of MAF, perturbing its activation and stability. Here, we characterize clinically and molecularly four additional patients. Expanding the series, we provide a more accurate delineation of the clinical phenotype, particularly focusing on the skeletal features characterizing this disorder. Beside midfacial hypoplasia and joints limitations, we report that delayed bone age, radio-ulnar synostosis, carpal/tarsal and long bone defects, and hip dysplasia variably occur in affected subjects. Consistent with this findings, by functional characterization of mutations using informative cell models, we provide first data documenting defective osteoblast and osteoclast differentiation and function.

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P11.12D
A not so rare insertion of a retrotransposon (SVA-F) in one of the major Bardet-Biedl gene (*BBS1*)

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Bardet Biedl syndrome is a ciliopathy with typical features including retinitis pigmentosa, polydactyly, cognitive impairment and renal failure. Mutations in 22 genes account for 80% of the cases. *BBS1* represents the highest fraction of patients with pathogenic variations including for 17% of all BBS patients the c.1169T>G, p.M390R variant. Among the unsolved cases in our cohort, several patients were heterozygous carriers of this specific recurrent mutation suggesting a second allele in *BBS1*. Targeted exome sequencing and mobile element insertion (MEI) detection (Mobster) was applied to 200 BBS samples. Whole genome sequencing, *de novo* assembly and SNP array analysis were performed to characterize the genomic events identified. We identified a few large exons deletions in *BBS1* gene. Interestingly, 4 families carried the c.1169T>G variation *in trans* of a hominid-specific retrotransposon insertion (SVA-F) in exon 13 of *BBS1*. We characterized this insertion in the general population (>400 samples screened), determined that its sequence of 2435 bp contains hallmarks of LINE1 mediated retrotransposition with a 5' truncation compared to the canonical SVA-F sequence. We confirmed our initial finding (Redin *et al*, 2012) that MEI is occurring in BBS. We describe 4 new families with the same SVA-F insertion in *BBS1*. Thus this insertion is now described in 5 families since the initial case reported by Tavares *et al* (2018). Such findings highlight the importance of using dedicated bioinformatics pipelines to identify all types of variations. Our PhD student is supported by a FRM grant (ECO20170637509).

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P11.14B
Adult phenotype of Beckwith-Wiedemann syndrome

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Background: Beckwith-Wiedemann syndrome (BWS) phenotype usually mitigate with age and data on adulthood are scanty. Our study aims at reporting phenotype evolution and health issues in adulthood.

Methods: 31 patients (14 males), aged 18-58 years (median 28.45) with BWS were enrolled.

Results: 14 patients had IC2-hypomethylation, 5 paternal UPD11, two IC1-hypermethylation, one 11p15.5 micro-duplication, 5 negative molecular tests and 4 were not tested. Final tall stature was present in 38%. Three patients developed nephroblastoma (2, 3 and 10 years, respectively); one hepatoblastoma (22 years); one acute lymphoblastic leukemia (21 years); one adrenal adenoma and testicular Sertoli cell tumour (22 and 24 years, respectively); and three benign tumours (hepatic haemangioma, uterine myoma and mammary fibroepithelioma). Surgery for BWS-related features was required in 84%. Despite surgical correction several patients presented morbidity and sequelae of BWS pediatric issues: pronunciation/swallow difficulties (n=8) due to macroglossia, painful scoliosis (n=4) consistent with lateralized overgrowth, recurrent urolithiasis (n=4), azoospermia (n=3) likely consequent to cryptorchidism, severe intellectual disability (n=2) likely related to neonatal asphyxia and diabetes mellitus (n=1) due to subtotal pancreatectomy for intractable hyperinsulinism. Three

patients (2 males) had healthy children (two physiologically conceived and one through ART).

Conclusions: adult health conditions in BWS are mostly consequent to pediatric issues, underlying the preventive role of follow-up strategies in childhood. Malignancy rate observed in early adulthood in this small cohort matches that observed in the first decade of life, cumulatively raising tumor rate in BWS to 20% during the observation period. Further studies are warranted in this direction.

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P11.15C

Branchio-Oculo-Facial Syndrome: a case report on an atypical case with broad intra-familial variability

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Introduction: Branchio-oculo-facial syndrome (BOFS) is an autosomal-dominant inherited disorder, related to mutations in *TFAP2A*. This gene encodes for the AP2-α protein, which plays a major role in human craniofacial development. While cardinal features of this syndrome commonly involve branchial skin defects, oculo-facial abnormalities, and hearing loss, a broad phenotypic variability can hamper the diagnosis in some atypical cases.

Case Presentation: We report two siblings and their mother, with a familial history of Pierre Robin sequence without cleft palate, coloboma, pseudo-cleft aspect of the upper lip, nasolacrimal duct stenosis and hearing loss, with a broad intra-familial variability. Focusing on the severe syndromic Pierre Robin sequence and ear dysplasia in one affected child, a gene panel of mandibulofacial dysostosis (*EFTUD2*, *POLR1A*, *POLR1C*, *POLR1D*, and *TCOF1)* was performed without identifying any mutation. A CGH array showed a 22q11.21 microduplication in one of the affected children, assessed as insufficient to explain the poly-malformative familial phenotype. After a second clinical evaluation, a diagnosis of branchio-oculo-facial syndrome was suggested based on the association of "pseudo-cleft" defects of the upper lip, coloboma, and nasolacrimal duct stenosis. Specific Sanger sequencing of *TFAP2A* in the index case revealed a predicted pathogenic c.532+2T>C variant (NM_003220.2) in a heterozygous state, supporting the diagnosis of BOFS.

Conclusion: This case report highlights the broad variability of BOFS phenotypic spectrum. However, it emphasizes some distinctive clinical features, which can be unrecognized by clinicians, though highly relevant to reach the accurate diagnosis.

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P11.16D

Novel patient with Contiguous *ABCD1/ DXS1375E* (*BCAP31*) Deletion Syndrome (CADDS) and review of the literature

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The contiguous *ABCD1/DXS1375E* (*BCAP31*) deletion syndrome (CADDS) was first reported in 2002. Up to date there are only 6 reported patients. These patients presented a similar phenotype with marked neonatal hypotonia, severe growth failure, failure to thrive, profound developmental delay and liver dysfunction leading to early death in the first year. We present a 7th patient with CADDS, a 2 months old boy who presented severe antenatal and postnatal growth retardation, failure to thrive, irritability, mild hypotonia, transient liver dysfunction with cholestasis and elevated liver enzymes. He also had exocrine and possible endocrine pancreatic deficiency which has not yet been described. Chromosomal microarray showed a 60kb Xq28 including the first three exons of the *BCAP31* gene, the entire *ABCD1* gene and part of the *PLXNB3* gene. *ABCD1* loss-of-function mutations lead to X linked-Adrenoleucodystrophy, a neurodegenerative condition with broad clinical variability. No cases have been described under the age of 2.7 years. And no liver involvement has been described. *BCAP31* loss-of-function mutations have been identified in 7 patients from 3 families, all boys with severe developmental delay, dystonia, deafness, central hypomyelination, growth retardation. No apparent chronic liver dysfunction was

described. Four of the patients died in the first years of life, either suddenly or during a febrile episode. The phenotype of CADDS patients seems distinctive from those with isolated *ABCD1* or *BCAP31* loss-of-function. Different hypotheses have been made to explain the severe liver phenotype of the deletion, however further studies are needed to conclude.

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P11.17A

A novel missense variant of MAP3K7 causes cardiospondylocarpofacial syndrome by dominant negative effect

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Cardiospondylocarpofacial syndrome (CSCF) is characterized by growth retardation, dysmorphic facial features, brachydactyly, vertebral synostosis, cardiac septal defects, and deafness.

Here we report a patient with growth retardation, dysmorphic facial appearance, vertebral abnormalities and congenital heart defect associated with a pathogenic variant in *MAP3K7* identified by whole exome sequencing (WES) and animals model analyses. The patient was a four-year-old boy. The boy was born at 39 weeks of gestation with apgar index of 3 (1 min) and 6 (5 min). After birth, the boy was soon administrated our hospital due to severe cyanosis. At 4 year-of-age, the patient showed short stature (-5 S.D.), developmental delay, dysmorphic facial features with macrocephalus, long face, frontal bossing, epicanthal fold and mid-face hypoplasia, coarctation of aorta and atrial septal defect, scoliosis, vertebral synostosis, and joint laxity. Written informed consent was obtained from his parents.

WES analysis was performed and detected variants were confirmed using Sanger sequencing. A novel heterozygous missense variant, c,574 A>G (p.S192G), in the *MAP3K7* gene was found in the patient. The variant was de novo.

We confirmed the effect of the variant using transgenic Drosophila. The *MAP3K7* gene with the variant was introduced into Drosophila overexpressed eiger (TNFalpha), which was reduced the eye-size. Then, the eiger effect was suppressed by the mutant MAP3K7, suggesting that the variant showed dominant negative effect. We concluded that the patient was caused by the missense de novo variant of *MAP3K7* and CSCF was caused by loss-of-function of MAP3K7.

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P11.20D

Deleterious mutations in one third of non-syndromic discontinuous cleft lip and palate patients

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Cleft of the lip and/or palate (CLP) are among the most common birth defects, with an approximate incidence of 1/700 live births and with a wide variability of expression depending on ethnicity, gender and cleft type. When cleft of the palate is associated with a cleft of the lip with preservation of the primary palate, it defines an atypical phenotype called *discontinuous cleft*. Although this phenotype may represent 5% of clefts of the lip and/or palate (CLP), it is rarely specifically referred to and its pathophysiology is unknown. To date only few WES studies, mostly applied to familial cases, have been performed to search for rare coding variants in non-syndromic CLP individuals. One study identified mutations in genes mutated in syndromic forms of CLP in 10%. We conducted Whole Exome sequencing (WES) on 8 non-syndromic discontinuous CLP individuals whether familial or not in order to identify genes and mutations that could underlie this phenotype. We discovered loss-of-function mutations in 3 out of the 8 individuals in 3 genes previously implicated in CLP, representing almost 40% of this cohort. Whole exome sequencing of clinically well-defined

subgroups of CLP, such as discontinuous cleft, is a relevant approach to study CLP etiopathogenesis. Non-syndromic discontinuous cleft lip and palate seems to have a strong genetic basis.

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P11.21A
A new cloverleaf skull syndrome in a pair of twins

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Cloverleaf skull (Kleeblattschaedel, OMIM 148800) is a rare congenital anomaly resulting from complex craniosynostosis. The condition shows clinical and etiologic heterogeneity. The cause of isolated cloverleafskull is unknown, and this feature is a component of at least 15 monogenic disorders which account for majority of all cloverleaf skull syndromes (JM Graham & PA Sanchez-Lara, 2016). A diamniotic, dichorionic pregnancy was assessed starting at 20 weeks gestation for skull and possible brain anomalies by ultrasound and MRI. An initial suspicion of encephaloceles in both fetuses was raised followed by that of craniosynostosis. Genetic testing was postponed for the postnatal time. The 24-year-old G2P1A1 mother experienced PPROM at 28 weeks gestation and delivery took place by C/S at 32w2d gestation. Both newborns, a boy and a girl shared the same phenotype including typical craniofacial dysostosis associated with cloverleaf skull, and bilateral parietal encephaloceles, micromelia, no skeletal anomalies, and unilateral kidney dysplasia in one fetus. Extensive genetic testing including targeted sequencing (FGFR1, 2, and 3, TWIST1), craniosynostosis panel, exome sequencing (WES) and chromosomal microarray (CMA) did not reveal a clear etiology. WES and CMA showed a 2.4 Mb VUS deletion at chromosome 18p11.32, seen in gnomAD, shared between the two affected newborns and the father who has a history of an unclear seizure disorder, mild developmental delay and no craniosynostosis. We are raising the possibility of a new genetic syndrome with cloverleaf skull of unknown etiology that requires further investigations to inform appropriate counseling for this young family.

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P11.22B
Adding evidence to the role of *NEUROG1* in congenital cranial dysinnervation disorders (CCDDs): a case report

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Introduction: CCDDs are a heterogeneous group of neurodevelopmental phenotypes caused by a primary disturbance of innervation due to deficient, absent, or misguided cranial nerves. Several phenotypes still await etiological elucidation.

NEUROG1 encodes Ngn1 which is a basic helix-loop-helix transcription factor essential for the formation of the trigeminal, vestibule-cochlear and accessory nerves. So far it hasn't been associated with a phenotype in OMIM. Nonetheless, in 2013 Schroder et al (PMID: 23419067) reported a patient with mild developmental delay (DD), bilateral profound sensorineural deafness due to absent bilateral VIII nerves, and severe oromotor dysfunction, who had a homozygous deletion including *NEUROG1*.

Case description: We describe a 10 year- old boy with hypotonia, mild DD, bilateral profound sensorineural hearing loss, and keratoconjunctivitis due to lack of corneal reflex and incomplete eye closure. On physical examination he had a long expressionless face, right eye leucocoria, and severe oromotor dysfunction. EMG showed axonal sensory polyneuropathy of the lower limbs. Brain-MRI revealed bilateral agenesis/severe hypoplasia of the VIII nerve with marked atresia of the internal auditory canals and cochlear labyrinth malformation. Trio-exome identified a homozygous variant in *NEUROG1* (NM_006161.2: c.202G>T, p.Glu68*). This variant is classified as uncertain according to ACMG guidelines, but is considered pathogenic applying the ClinGen SVI Bayesian classification framework (posterior probability 0.997).

Conclusions: The resemblance between our case and Schroder's is remarkable. This case adds support to establishing *NEUROG1* as a new gene for CCDDs associated with a very distinctive phenotype, and contributes to a better understanding and classification of these disorders.

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P11.23C
Genomic overlap between neurodevelopmental disorders and congenital heart defects

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Introduction: The phenotypic overlap between neurodevelopmental disorders (NDD) and congenital heart disease (CHD) is well documented and in this study, we investigated the genomic overlaps in large clinical cohorts.

Materials and Methods: We analyzed whole genome clinical microarray data from 10,797 NDD and 3,174 CHD cases from Ontario to analyze large copy number variations (CNVs). Clinical cytogeneticists identified a consensus set of variants(pathogenic, variant of uncertain significance (VUS)) based on established guidelines. We have conducted pathway enrichment and brain single cell trancriptome analysis to identify cell types for pleiotropic genes that are impacted in both NDD and CHD cases.

Results: We have identified 186 *de novo* CNVs from NDD and CHD cases. Our analysis on clinically relevant CNV revealed 573 and 181 pathogenic variants in NDD and CHD, respectively. Similarly, we have identified 4753 and 1361 variants of uncertain significance in NDD and CHD, respectively. Genes with at least one exon impacted by pathogenic(OR=6.41) and VUS(OR=6.68) deletions shows significant (P<0.001) overlaps between the NDD and CHD cases. Similar significant overlap was also observed for duplications. "activation of gtpase activity" pathway showed significance (P<0.001) for the overlapping genes. For example, *CLSTN1* gene was found to be impacted by pathogenic variants for both NDD and CHD. Single cell analysis shows overlapping genes are highly expressed in neurons and astrocytes.

Conclusions: We have identified significant overlap of pathogenic and VUS CNVs between NDD and CHD samples. This study also shows evidence of specific cell types that might contribute to the etiology of NDD and CHD.

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P11.24D
49 novel recessive candidate genes for intellectual disability and visual impairment in 350 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. In Pakistan, the frequency of consanguineous marriages approaches 70%. To bridge the gap between the 1800 known and the estimated >9000 recessive gene-phenotypes, we have initiated a Swiss-Pakistani project to identify novel recessive candidate genes for two phenotypes: Intellectual Disability (ID) and Visual Impairment

(VI). We have collected samples from 1265 individuals of 197 ID, and 1809 individual of 236 VI families of first cousin marriages with at least two affecteds. Exome sequence of one affected and genotyping of the whole family (parents, all affected and unaffected siblings) has been completed in 166 ID and 184 VI families to date. The likely causative gene/variant in known genes was found in 64% of the VI and 32% in the ID families. Thus, there are more unknown "recessive genes" for ID. In 18% of the VI families we have identified 25 novel candidate genes, and in 27% of the ID families 24 such candidates (to be presented in the conference). International genematching identified additional families in 20% of the candidate genes. Careful evaluation of the phenotypes is mandatory to assess the possibility of two or more causative genes in certain families, and to minimize false negative results. International databases from consanguineous individuals are needed to facilitate the assignment of pathogenicity to homozygous variants.

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P11.25A

A CNV positional effect analysis implicates enhancer-mediated *SHH* dysregulation in a patient with multiple congenital anomalies and malformations

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Introduction: Despite application of genome-wide diagnostics, such as chromosomal microarrays and whole exome sequencing, a significant proportion of patients with suspected genetic disease continue to lack a definitive diagnosis. The underlying genetic defects in a subgroup of these cases may be altered expression of disease genes due to copy-number variants (CNV) involving regulatory non-coding elements. Therefore, in a cohort of cases with suspected genetic diseases who underwent chromosomal microarray analysis, we searched for CNV interposed between relevant disease genes and their corresponding enhancers.

Methods: Out of a dataset of 1,176 CNV from 682 patients, we selected CNVs that were: (1) smaller than 1 Mb, (2) devoid of disease genes, and (3) mapped between disease genes and their enhancers. Then, we manually analyzed these *gene-CNV-enhancers* groups for concordance between involved genes and corresponding phenotypes. Finally, we predicted effect of CNV on chromatin folding by the strings-and-binders method.

Results: 235 CNV (34%) mapped between a disease gene and one of its enhancers and 26 gene-phenotype relationships were consistent after manual evaluation. Among these cases, the best CNV candidate was a 50 kb de novo deletion encompassing a region between sonic hedgehog gene (*SHH*) and two of its enhancers, in a patient with limb abnormalities, aortic malformation, cardiac arrythmia, and facial dysmorphisms.

Conclusion: Systematic analysis of potential positional effect of CNV has potential for identifying the genetic defects in undiagnosed cases and in our analysis it detected a CNV affecting contacts of *SHH*, an important transcription factor involved in development, with its enhancers.

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P11.28D

Down syndrome-iPSC neurogenesis connects differential methylation to dysregulated gene expression

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Introduction: Down syndrome is caused by trisomy 21 (T21) in humans and affects approximately one in 700 live births. Despite major efforts the molecular mechanisms leading to the morphological and functional brain abnormalities associated with T21 remain largely unknown.

Material and Methods: To clarify the role of differential methylation on transcriptional dysregulation and neurodevelopment in T21, we established an induced pluripotent stem cell (iPSC) derived neural cell model showing a transctiptional profile comparable to early-mid gestational

period. The DNA methylation pattern was analysed using Illumina HumanMethylation 450k BeadChip and paralleled by RNA sequencing.

Results: We assessed the genome wide methylation pattern in T21 and euploid iPSC neural derivatives and identified 500 differentially methylated positions (DMPs). Approximately half of DMPs (281 of 500) could be annotated to a total of 202 genes. Gene Ontology (GO) analysis of these 202 genes revealed enrichment of neurotransmitter transporters (GO:0006836). Integrated analysis of methylation and transcriptome data sets revealed altered expression in 77 out of the 202 genes in T21 lines. Furthermore, the most profound methylation changes (>9DMPs/gene) associated with differential expression was observed for a cluster of genes (*ZNF69* and *ZNF700*) on chromosome 19 encoding zink finger transcription factors. These *ZNF* genes have yet unknown functions and are highly expressed during normal embryonic brain development.

Conclusion: Our results suggest that differential methylation contributes to transcriptional dysregulation in T21 neural cells derived from iPSCs. Our study further highlights a set of *ZNF* transcription factor genes showing profound differential methylation in T21 neurogenesis.

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P11.29A

DPH1 syndrome. Clinical review and structural and functional analyses of seven disease-causing variants identified so far

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Introduction: DPH1 variants have been associated with an ultra-rare and severe neurodevelopmental disorder mainly characterized by variable developmental delay, short stature, dysmorphic features, and sparse hair.

Material and methods: WES was applied to two trios with undiagnosed patients. A survey of all the clinical features of 17 DPH1 syndrome patients from 7 families was performed. The DPH1 enzyme activity of wild-type and of 7 disease-causing mutants was assessed through the diphtheria toxin ADP-ribosylation assay. A homology model of the human DPH1-DPH2 heterodimer was built, and molecular dynamics simulations were performed to study the effect of these variants on the catalytic sites, as well as on the interactions between subunits of the heterodimer.

Results: Two DPH1 novel variants were identified in 2 independent families, enriching the clinical delineation of DPH1 syndrome. The enzyme assay demonstrated compromised functionality for 5 mutations (p.Leu234Pro; p.Ala411Argfs*91; p.Leu164Pro; p.Leu125Pro; p.Tyr112Cys). According to the structural model, p.Leu125Pro may affect dimerization while p.Tyr112Cys, p.Leu164Pro, p.Leu234Pro and p.Pro382Ser may interfere the binding of the iron-sulphur cluster necessary for catalysis.

Conclusions: The overall good correlation observed between DPH1 protein activity, structural prediction and clinical features indicate that these biochemical and structural tests may be useful tools for assessing the pathogenicity of DPH1 variants and for helping to predict the clinical severity of future DPH1 cases.

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P11.30B

Pontocerebellar hypoplasia with rhombencephalosynapsis and microlissencephaly expands the spectrum of PCH type 1B

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Rhombencephalosynapsis (RES) is a rare cerebellar malformation, developing during embryogenesis, defined by complete or partial vermis agenesis with fusion of the cerebellar hemispheres. It occurs either alone or in association with other cerebral and/or extracerebral anomalies. Its association with microlissencephaly is exceedingly rare and to date, only a heterozygous *de novo* missense variant in *ADGRL2*, a gene encoding Adhesion G-Protein-Coupled Receptor L2, has been identified. Here, we report two siblings of Roma origin presenting with severe growth retardation, fetal akinesia, microlissencephaly and small cerebellum with vermian agenesis. Neuropathology showed extreme paucity in pontine transverse fibres, rudimentary olivary nuclei, RES and vanishing motoneurons in both foetuses. Comparative foetus-parent exome sequencing revealed in both foetuses a homozygous variant in exon 1 of the *EXOSC3* gene encoding a core component of the RNA exosome, c.92G>C;p.(Gly31Ala). *EXOSC3* variants account for 40% to 75% of patients affected by ponto-cerebellar hypoplasia with spinal muscular atrophy (PCH1B). The c.92G>C variant is a founder mutation in the Roma population and has been reported in severe PCH1B. PCH1B is characterized by a broad phenotypic spectrum, ranging from mild phenotypes with spasticity, mild to moderate intellectual disability, distally pronounced muscular atrophy, and cerebellar atrophy, to severe phenotypes with profound global developmental delay, progressive microcephaly, and atrophy of the cerebellar hemispheres. The typical dragonfly pattern of the cerebellum observed in PCH1B patients with flattened hemispheres and relative prominence of vermis differs markedly from RES. This novel foetal presentation expands the spectrum of PCH1B and highlights the diversity of RES aetiologies.

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P11.31C
MR neuroimaging and EEG findings in 62 patients with Fetal alcohol spectrum disorders

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Introduction: The fetal alcohol spectrum disorders (FASD) span a group of neurodevelopmental disorders related to maternal alcohol intake during pregnancy. Studies correlating EEG and neuroimaging with specific FASD categories are lacking.

Material and methods: Classification of 62 patients into 3 FASD categories, including fetal alcohol syndrome (FAS), partial FAS (pFAS) and alcohol related neurodevelopmental disorders (ARND). Prospective studies of MR imaging and EEG.

Results: Neuroimaging: The most frequent findings were corpus callosum abnormalities (42%) and cerebellar vermis hypoplasia (24%). Additional findings were vascular anomalies, gliosis, prominent perivascular spaces, occipito-cervical junction and cervical vertebral anomalies, pituitary hypoplasia, arachnoid cysts, and cavum septum pellucidum. Only 16% had normal neuroimaging. EEG (including sleep recording in 43%) showed anomalies in 23%, including slowing of background activity and interictal epileptiform discharges, focal and/or generalized, and 3 of them had epilepsy. In one patient, seizures were first detected during the EEG recording and one case had an encephalopathy with electrical status epilepticus during slow sleep (ESES). Focal interictal discharges in our patients did not imply the presence of underlying visible focal brain lesions in the neuroimaging studies, such as cortical dysplasia or polymicrogyria. However, they had nonspecific brain MR abnormalities, including corpus callosum hypoplasia, vermis hypoplasia or cavum septum pellucidum. The latter was significantly more frequent in the group with EEG abnormal findings ($p < 0.01$).

Conclusion: Patients with FASD exhibit frequent and diverse neuroimaging and EEG findings.

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P11.32D
Gating-affecting mutations in *KCNK4* cause a recognizable neurodevelopmental syndrome

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Introduction: Aberrant activation or inhibition of potassium (K+) currents across the plasma membrane of cells has been causally linked to altered various functions in both excitable and non-excitable cells. KCNK4 belongs to the mechano-gated ion channels of the TRAAK/TREK subfamily of two pore-domain (K2P) K+ channels. While K2P channels are well-known to contribute to the resting membrane potential/cellular excitability, their involvement in pathophysiological processes remains largely uncharacterized.

Materials and Methods: In the frame of the Undiagnosed Patients Program at the Ospedale Pediatrico Bambino Gesù, Rome, the exomes of two unrelated subjects with a molecularly unexplained, clinically superimposable phenotype were scanned to identify the underlying molecular cause. Patch-clamp analyses, co-expression experiments and molecular dynamics simulations were used to functionally characterized the genomic findings.

Results: Two variants in *KCNK4* were identified as the only shared events in these two patients and a third was found by using GeneMatcher. Patch-clamp analyses documented an impressive gain-of-function of the identified KCNK4 channel mutants. Co-expression experiments provided evidence of the dominant behavior of the disease-causing mutations. Remarkably, molecular dynamics simulations consistently indicated that mutations seal the lateral intramembrane fenestration proposed to negatively control K+ flow.

Conclusions: We report that *de novo* missense mutations in *KCNK4* cause a recognizable syndrome for which we propose the acronym FHEIG (facial dysmorphism, hypertrichosis, epilepsy, intellectual disability/developmental delay, and gingival overgrowth). Overall, our findings illustrate the pleiotropic effect of dysregulated KCNK4 function and provide support to the hypothesis of a gating mechanism based on the lateral fenestrations of K2P channels.

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P11.33A
De novo mutation of *CSNK2B* encoding beta subunit of casein kinase 2 causes Filippi syndrome

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Introduction: Filippi syndrome (FS) is a rare genetic condition characterized by short stature, microcephaly, intellectual disability, syndactyly and distinctive facial features. A total of 33 patients from 25 families are documented worldwide. Previously, we reported the first gene (*CKAP2L*) involved in FS. Here, we ascertain a

mutation in *CSNK2B* as a likely cause of FS. *CSNK2B* encodes CK2β – the beta subunit of casein kinase 2, which plays pivotal role in diverse cellular processes.

Material and Methods: To identify the causal variant, trio whole-exome sequencing was performed. To further explore the consequences of the mutation, pulldown assays, microscale thermophoresis (MST), immunofluorescence and RNA-seq transcriptome profiling of lymphoblastoid cell lines (LCLs) were conducted.

Results: A pathogenic *de novo* mutation (*CSNK2B:* c.94G>C; p.Asp32His) was identified in an Italian patient diagnosed with FS. The mutation caused an up-regulation of *CSNK2B* expression at transcript and protein level, which resulted in impaired cross talk between α and β subunits of CK2. The effects of mutation were also observed in two crucial pathways; canonical Wnt signaling (CWS) and DNA damage response (DDR). In CWS, an impaired interaction of DVL3 with mutant CK2β and up-regulation as well as mislocalization of β-catenin in mutant LCLs was found. In DDR, γH2AX was substantially increased in mutant LCLs. Finally, RNA-seq data confirmed differential expression of proteins involved in the aforementioned pathways.

Conclusion: Our findings suggest that *CSNK2B* is a novel candidate gene for Fillippi syndrome and the mutation found in our patient causes the disorder by dysregulating CWS and DDR.

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P11.34B
Intestinal elongation and motility require the long isoform of FLNA

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Introduction: *Filamin A* (*FLNA*) encodes a cytoskeletal protein that regulates cell shape by cross-linking actin filaments. Mutations in *FLNA* have been associated with a wide spectrum of disorders, and more recently with an X–linked form of Congenital Short Bowel Syndrome (CSBS). These mutations are located between two methionines present at the N-terminal of the protein, and seem to only block expression of the long isoform of FLNA.

Material and Methods: To understand the role of FLNA in intestinal development, expression studies at different human embryonic stages, and several *in vitro* studies were performed. A transgenic zebrafish line was also generated using TALENs, where expression of the long isoform of FLNA was specifically blocked.

Results: FLNA expression was detected in the muscular layer of the small intestine from early embryonic stages. Moreover, *FLNA* mutations associated with CSBS, blocked expression of the long isoform of FLNA, but did not affect the binding of FLNA to actin filaments. These mutations also impaired contractility of human intestinal smooth muscle cells *in vitro*. Finally, *FLNA* mutant fish were phenotypically indistinguishable from wild-type fish, except for a significant reduction in gut length (10%), and diminished intestinal motility.

Conclusion: Our results bring new insights into CSBS pathogenesis, by showing that the intestinal defects associated with this disease, are likely caused by impaired smooth muscle contraction, as a consequence of the loss of expression of the long isoform of FLNA.

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P11.35C
Intragenic *de novo* frame shift deletion of two exon confirms a role for *FNDC3B* in human craniofacial development and possibly neuro-psychiatric development

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Background: De novo 3q26.31 microdeletions has been reported in four cases with dysmorphic facial features. The overlapping deleted region was shown to only contain the FNDC3B gene. The product of this gene, the fibronectin domain III-containing protein 3B (also known as factor for adipocyte differentiation-104 (FAD104)) has been identified in mouse models as a positive regulator of adipocytes differentiation but also as a negative regulator of osteoblast differentiation. Cranial changes have also been described for *FNDC3B* knock-out mice.

Methods: Agilent 180K oligo array CGH analysis and clinical evaluation including a neurodevelopmental evaluation.

Results: We report the first case of a *de novo* intragenic microdeletion involving two exons of the FNDC3B gene resulting in an frameshift deletion. The patient is a boy with subtle dysmorphic facial features and behavioral problems

within the autism spectrum. The boy is 9 years old and we present the developmental evaluation at different ages.

Conclusion: CNVs have contributed much to our understanding of disease development and the underlying mechanism. However, the role of individual genes in disease development can be difficult to interpret in cases where the deletion includes more genes or expands beyond the boundaries of a single gene. Such deletions may include crucial elements that affects the 3 dimensional structure of the genome and therefore affects the function of several genes in the region. This is the first case with an intragenic exon deletion in *FNDC3B* supporting a role for this gene in both craniofacial development and neuro-psychiatric development.

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P11.36D

Genetic screening of Noonan syndrome in central north of Morocco: achievements, challenges & perspectives

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Introduction: Noonan syndrome is an autosomal dominant disorder with an incidence of 1/1000 - 2500 live births. While the very first genetic test in Morocco was carried out in the nineties, the first genetic diagnosis of Noonan syndrome (NS) was performed seven years ago. Thus, the aim of this presentation is to expose results of molecular screening of Noonan syndrome in the central north of Morocco, and discuss its challenges and perspectives.

Material and methods: Thirty-one patients were recruited in the medical genetics laboratory from different regions of central North of Morocco. After obtaining informed consents, genomic DNAs were extracted from leucocytes. Then, all samples were screened for PTPN11 mutations using PCR and direct sequencing. The obtained sequences were analyzed using NCBI bioinformatics tools.

Results: We have detected five pathogenic missense mutations, one synonymous mutation and three novel intronic duplications. All variants were heterozygous. The pathogenic mutations were clustered on exons three and eight, while most of the non-coding variants were localised in the intron four. Considering only the pathogenic mutations, PTPN11 mutation rate in our cohort was around 16%.

Conclusions: Since the first diagnosis of PTPN11 mutations in a Moroccan Noonan family, considerable efforts have been made to improve the genetic services offered to patients. Although several challenges are still retarding the progress of NS genetic diagnosis in Morocco, the NGS technologies that have recently been installed seems to provide a promising future.

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P11.37A

Does my expertise still make a difference? A single-clinician's experience of genomic sequencing in 120 pediatric patients

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Introduction: Genomic sequencing (WES/WGS) provides diagnosis in 40% of unsolved cases in the pediatric population. Current clinical approach mandates integration of phenotypic data with variant analysis. This study aims to assess the diagnostic yield of NGS ordered by a single clinician to a single laboratory.

Materials and Methods: Data from 120 patients were retrospectively analyzed. Time interval was September 2015-January 2019. Tests were performed at Centogene, Germany. All patients were re-evaluated after test results. Variants of unknown significance (VUS) were specifically re-analyzed with evolving clinical story, current literature and results of other investigations. Diagnoses confirmed by NGS (ACMG Class 1 or 2 variants) were coined as "suggested molecular diagnosis". Those established after clinical re-analysis were considered "robust clinical diagnosis".

Results: Median age was 4.7 (0.1-15.1) years. M/F ratio was 1.14. Time passed since first symptom until a robust diagnosis was 2.8 (0.1-14.3) years. Blended phenotype was present in 8%. Table 1 summarizes the data. Diagnostic rate of trio-based WES was significantly higher than proband-only WGS, where both increased after clinical re-evaluation. Suggested molecular diagnosis was significantly higher in trio-based testing. Consanguinity,

	Number of index patients	Suggested molecular diagnosis		Robust genetic diagnosis		Difference between molecular and robust diagnosis		No diagnosis (%)
		Positive (%)	p value	Positive (%)	p value	Percentage	p value	
Test type			**0.008**		0.779		**0.054**	
Proband-only WES	13	7 (54%)		10 (77%)		23%		3 (23%)
Trio-based WES	59	27 (46%)		42 (71%)		25%		17 (29%)
Proband-only WGS	41	10 (24%)		28 (68%)		44%		13 (32%)
Trio-based WGS	7	6 (86%)		6 (86%)		0%		1 (14%)
Test type (proband-only vs trio-based)			**0.041**		0.776		0.055	
Proband-only	54	17 (32%)		38 (70%)		39%		16 (30%)
Trio-based	66	33 (50%)		48 (73%)		23%		18 (27%)
Consanguinity			0.647		0.717		0.891	
No	89	36 (40%)		63 (71%)		30%		26 (29%)
Yes	31	14 (45%)		23 (74%)		29%		8 (26%)
Affected siblings			0.508		0.856		0.593	
No	100	43 (43%)		72 (72%)		29%		28 (28%)
Yes	20	7 (35%)		14 (70%)		35%		6 (30%)
Dysmorphic features			0.666		0.861		0.524	
No	58	23 (40%)		42 (72%)		33%		16 (28%)
Yes	62	27 (44%)		86 (72%)		27%		18 (28%)

affected siblings and dysmorphic features did not have an effect on diagnostic rate.

Conclusions: Our results emphasize the additional impact of clinical expertise. An experienced clinician familiar with rare disease diagnoses is essential to determine whether a variant is causative, contributory or unrelated.

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P11.38B

Flow-cytometric analysis confirms the GPI biosynthesis deficit in a family with an atypical phenotype associated with a *PIGA* variant

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This study aimed to identify the underling molecular defect in a family with two male adults showing a similar phenotype consisting of distinct facial features, intellectual disability with a complete lack of speech, hypotonia, in combination with abnormal muscle, skin, urinary, and dental findings. Exome sequencing (ES) was carried out on one of the patients. Functional validation was performed by flow cytometry determining surface expression of GPI (identified by FLAER) and GPI anchored proteins (GPI-AP) in white and red blood cells. ES identified a hemizygous variant in *PIGA* gene: NM_002641.3: c.232A>G; p.(Lys78Glu). Sanger sequencing confirmed the presence of the variant in both brothers and established that the mother is a carrier. Flow cytometry results showed that, despite normal peripheral blood counts, patient granulocytes/neutrophils and monocytes were partially deficient in cell surface GPI and at least some GPI-AP (*e.g.* CD16 in neutrophils and CD14 in monocytes), supporting the pathogenicity of the *PIGA* variant. In contrast, red blood cells had normal levels of GPI-AP (CD55 and CD59). The

p.(Lys78Glu) missense mutation results in a partial deficiency of GPI and GPI-AP affecting the myeloid, but not the erythroid cells. Notably, the amino acid substitution described herein leads to diminished expression of CD14 on monocytes as well, representing the first monocyte defect described in a germline *PIGA* case. Funding: UMIB is supported by National Funds through the FCT - Fundação para a Ciência e a Tecnologia in the frameworks of the UID/Multi/0215/2016 project. Grants: NM, DEFI-CHUP, EPE PhD 2015 and PJ, research grant 145/2015.

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P11.39C

Severe haemophilia A caused by an unbalanced chromosomal rearrangement identified using nanopore sequencing

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Background: No *F8* genetic abnormality was detected in about 2% of severe haemophilia A patients using conventional genetic approaches. In these patients, deep intronic variation or *F8* disrupting genomic rearrangement could be causal.

Objective: to characterize a Xq28 rearrangement disrupting *F8* in a genetically unresolved severe haemophilia A patient.

Materials and Methods*:* a large panel of comprehensive molecular techniques including long-range PCR, RNA analysis, nanopore sequencing and cytogenetic analysis were used.

Results: Long-range PCR performed throughout *F8* identified a non-amplifiable region in intron 25 indicating the presence of a chromosomal rearrangement. *F8* mRNA analysis including 3'Rapid Amplification of cDNA Ends and nanopore sequencing showed the presence of a *F8* fusion transcript in which *F8* exon 26 was replaced by a 742 bp pseudo-exon corresponding to a non-coding region located at the beginning of the long arm of chromosome X (Xq11.1). The pseudo-exon was flanked by a canonical AG on its 5'side and contained two putative polyadenylation signals on its 3'side. Cytogenetic Microarray Analysis showed the presence of a 3.8 Mb gain of the Xq11.1q12 chromosomal region. PCR amplification of junction fragments and FISH analysis confirmed that the Xq11.1q12 duplicated region was inserted in *F8* intron 25.

Conclusion: Exceptionally, this structural variant characterization was conducted from cDNA analysis. To our knowledge, this is the first case of chromosomal rearrangement revealed through nanopore sequencing without previous cytogenetic study. This study highlights the usefulness of single molecule long-read sequencing technologies for molecular diagnosis of genetic disorders especially when structural variants are suspected.

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P11.40D

Modelling Hirschsprung disease with human induced pluripotent stem cells: a preliminary study

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Introduction: Hirschsprung disease (HSCR) is a congenital neuropathy characterized by the lack of the enteric nervous system in the distal part of the colon. Mutations in more than 20 genes have been identified. One single good *in vitro* model to determine the functional consequences of the different mutated genes is lacking. In this study we tested whether induced pluripotent stem cells (iPSCs) could serve as such a model.

Material and Methods: Functional assays of the *EDN3*, *EDNRB*, and *ZEB2* variants identified, were performed by overexpressing the corresponding variant in HEK293 cells. qRT-PCR and Western Blot were performed to investigate the effect of these mutations on protein expression. In parallel, five iPSC lines obtained from fibroblasts derived from four HSCR patients carrying the mutations tested, and one individual control were differentiated into Enteric Neural Crest Cells (ENCCs) according a previous described protocol. ENCCs differentiation was evaluated using FACS and immunostaining.

Results: The *EDN3* mutant showed lower expression in comparison to the wild type, while the variants in *EDNRB* and *ZEB2* had the opposite effect. iPSC-derived ENCCs were positive for the neuronal marker HNK1. However, patient derived iPSCs yield lower percentage of ENCCs than controls.

Conclusions: The mutations identified in *RET*, *GFRA1*, *EDN3*, *EDNRB* and *ZEB2* showed significant differences on protein expression, and seem to impair iPSCs differentiation into ENCCs. However, further studies are needed to fully characterize the effect of these mutations on the iPSC-derived ENCCs to show whether indeed iPSCs are the perfect model system for HSCR.

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P11.41A

Screening for PALM2 and AKAP2 mutations in a set of congenital hypogonadotropic hypogonadism patients: a case with a chromosomal deletion at 9q31, PALM2 and AKAP2 chromosomal region

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Introduction: The genetic cause of congenital hypogonadotropic hypogonadism (cHH) remains unidentified in half of the patients. PALM2 encodes paralemmin-2, a paralemmin family plasma membrane protein, and AKAP2 encodes an A-kinase anchor protein. PALM2 and AKAP2 are adjacent on chromosome 9 and can form fusion transcripts. PALM2 has been implicated in cHH, since a deleterious PALM2 mutation has been found in one cHH patient. A chromosomal translocation causing monoallelic expression of AKAP2 has been found in another patient with Kallmann syndrome (cHH and anosmia/hyposmia) and bone anomalies.

Materials and Methods: Targeted sequencing of ANOS1, CHD7, FGF8, FGFR1, GNRHR, KISS1R, PROK2, PROKR2 and TACR3 and subsequent whole-genome linked-read sequencing was performed on a patient with cHH, anosmia, learning difficulties and motoric problems. We Sanger-sequenced the coding regions and exon-intron boundaries of PALM2 and AKAP2 in 22 Finnish cHH patients who have no mutations in currently known cHH genes.

Results: The patient carried a 2.3 Mb chromosomal deletion at 9q31.2 that excludes PALM2/AKAP2. Targeted sequencing revealed no other causative mutations or novel rare variants in this proband. In our patient set, no likely causative mutations were found.

Conclusions: We report a patient with a deletion at 9q31.2 yet intact PALM2 and AKAP2 genes. The roles of PALM2 and AKAP2 in Kallmann syndrome require further investigations.

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P11.42B

Overgrowth disorders with intellectual disability due to mutations in chromatin regulatory genes

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Introduction: Overgrowth disorders (OGS) encompass an heterogeneous group on conditions in which the main features is an increase of weight, height and head circumference or a combination of all, above +2SD for age, sex and ethnicity. Most OGS has additional features associated such as intellectual disability. Thus, the aim of this project was to perform a molecular screening of OGS with ID and to understand the underlying physio pathogenic associated mechanisms.

Material and Methods: Patients were selected retrospectively from the Spanish Overgrowth registry database (SOGRI). Molecular analysis included Sanger sequencing, MLPA and a custom NGS panel of 211 genes (Overgrowth v2.3). Variants annotation and prioritization was performed with a custom in-house bioinformatic script.

Results: A subgroup of patients with OGS+ID caused by mutations in chromatin regulation genes was observed: (5) *BRWD3* (X-linked mental retardation), (6)*DNMT3A* (Tatton-Brown-Rahman syndrome), (2)*SETD2* (Luscan-Lumish syndrome), (3)*EZH2* (Weaver syndrome), (99)*NSD1* (Sotos syndrome).

Conclusions: There is a high relationship between patients who present some overgrowth disorders with intellectual disability, with alterations in chromatin regulatory genes. These genes encode enzymes that possess histone methyltransferase activity, and therefore, are critical during the processes of human development. A correct molecular and clinical characterization is essential to carry out an adequate advice in patients with overgrowth that associate other clinical characteristics that overlap with several entities.

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P11.43C

MRX93 syndrome (BRWD3 gene): five new patients with novel mutations

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Introduction: Overgrowth syndromes (OGS) comprise a heterogeneous group of disorders whose main characteristic is that either the weight, height, or head circumference are above the 97th centile or 2-3 standard deviations (SD) above the mean for age and sex. Additional features are usually associated with OGS. Genetic analysis in patients with overlapping clinical features is essential, in order to distinguish between two or more similar conditions, and to provide appropriate genetic counseling.

Material and Methods: Patients were selected from the "Spanish Overgrowth Registry" (SOGRI). All patients or tutors gave informed consent. Two targeted custom gene panels were designed (183genes, OGLYVAS V1.0) and (212genes, Overgrowth v2.3) respectively. After the filtering of the relevant variants and validation, classification and interpretation of the variants were made according to ACMG guidelines.

Results: Four different pathogenic variants have been found in five patients. Three of the four variants were classified as pathogenic and the missense variant as likely pathogenic. All variants were located within the WD40 and Bromodomain of the protein, and all was not previously reported in the 14 patients with MRX93 already published.

Conclusions: MRX93 syndrome (XLID due to BRWD3 mutations) is a relatively new and uncommon condition mainly characterized by ID, overgrowth and facial dysmorphism. We report five patients with novel variants in *BRWD3* and review all the published cases so far. MRX93 diagnosis should be suspected in male patients with these features and genetic testing is highly recommended in order to confirm the diagnosis.

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P11.44D

Interstitial 2q24.2q24.3 microdeletion: description of two new cases and delineation of the critical minimal region. A new emerging syndrome?

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Interstitial deletions in 2q24.2q24.3 are rare events reported in literature and in publicly available databases associated mainly with developmental delay and intellectual disability. The breakpoint of these deletions varies, extending from 7.5 Mb to 2.3 Mb. Although some clinical features seem to be recurrent, these deletions are associated with a clinically heterogeneous phenotype. This is probably due to the different deletion size and gene content, to genetic variation in the remaining allele and to the influence of the genetic background of the rest of the genoma. Here we report on two patients with similar clinical phenotype and interstitial 2q24.2q24.3 deletions of different size overlapping a 1.7 Mb region. In particular, patient 1 was characterized by hypotonia, growth retardation, and psychomotor developmental and language delay, dysmorphisms and hearing loss with a de novo 3,7 Mb 2q24.2q24.3 deletion. Patient 2 presented with hypotonia, psychomotor and language delay, stereotypes movements and epilepsy with a de novo 2,9 Mb 2q24.2q24.3 deletion. We identified the smallest region of

overlap encompassing eight genes and our attention was particularly drawn to *SLC4A10, DPP4, KCNH7*. All these three genes are associated to neurological features. *SLC4A10* is highly expressed in the cerebral cortex and hippocampus, where it regulates the neuronal intracellular pH. Abnormal levels of *DPP4* were seen in patients with neuropsychiatric disorders. *KCNH7* encodes a voltage gated potassium channels and is widely expressed in human central nervous system. In conclusion, haploinsufficiency of these genes may be a good candidate for the main clinical features of this emerging syndrome.

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P11.45A

Inversion-duplication-deletion of chromosome 8p: genotype-phenotype correlation and determination of a new minimal duplicated region involved in ACC in 29 patients

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Introduction: Interstitial inverted duplication 8p associated with a distal deletion of the short arm of chromosome 8 (invdupdel[8p]) is a complex and rare chromosomal rearrangement. Intellectual disability (ID) is constant, and anomalies of the corpus callosum (ACC) are present in 80% of (invdupdel[8p]) patients. Almost all reported patients carry the same 8pter deletion, but the size and proximal breakpoint of the duplication are variable. Previous studies suggested a correlation between the size of the duplication and the severity of ID. Moreover, Sajan et al. (2013) defined a minimal duplicated critical region of 10.7 Mb associated with ACC. However, no gene of ACC has yet been clearly identified in this region. In order to refine genotype-phenotype correlation in this complex rearrangement, we report clinical and chromosomal data from 29 new patients with invdupdel[8p].

Patients and Method: 3 fetus and 26 patients (mean age: 9.7 years) were included. CGH/SNP array were performed for all of them.

Results: All living patients (n=26/26) had developmental delay and ID (n= 5/22 mild and n=17/22 moderate to severe). 16/22 patients had ACC (4 complete agenesis, 7 partial agenesis, and 5 dysplasia), associated with other cerebral abnormalities in 11 patients. Mean size of the deletion was 6.9 Mb and duplication was 19.2 Mb. Discussion - Conclusion: As expected, the severity of ID is correlated with the size of the duplication. Our study refines the minimal duplicated critical region for ACC to a 1.7 Mb-long region spanning one gene only, which is a new candidate gene for ACC.

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P11.46B

KMT2A mutations (Wiedemann-Steiner Syndrome): a new phenotype of corpus callosum agenesis without intellectual disability

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Introduction: *KMT2A* mutations are responsible for Wiedemann-Steiner Syndrome (WDSTS), a rare autosomal dominant disorder characterized by dysmorphic features, hypertrichosis, short stature, and intellectual disability (ID). Corpus callosum anomalies have been described in 16% of patients. We report here 2 unrelated patients with a de novo *KMT2A* heterozygous variant presenting with isolated prenatal agenesis of the corpus callosum (ACC), and a normal psychomotor development. Patients: Patient 1: partial ACC was diagnosed prenatally. At 4 years and 6 months of age, he had normal psychomotor development, whereas attention and praxic disorders were observed, especially in fine motor skills. The WPPSI-III at 4 years ½ showed a heterogenous profile. In addition, he had postnatal weight growth retardation (-2SD) and dysmorphic features. Patient 2: ACC was diagnosed prenatally. At the age of 8 years, he had normal neurodevelopment. WPPSI-IV performed at 5 years was in a normal range, with a heterogenous profile. Mild dysmorphic features were observed.

Methods: Trio-based Whole Exome Sequencing (WES) was performed in the 2 male patients and their non-consanguineous and unaffected parents.

Results: Patient 1: WES analysis detected a novel *de novo* heterozygous missense variant in *KMT2A* (c.4256G>A; p.Gly1419Asp), predicted as probably damaging by all prediction tools. Patient 2: WES analysis detected a *de novo* heterozygous truncating variant in *KMT2A* (c.1539del; p.Ile515Phefs*52).

Conclusions: These two observations expand the WDSTS phenotypic spectrum, since ID may be absent. Thus, this syndrome might be considered in cases of isolated corpus callosum anomalies, associated with normal development.

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P11.47C

Expansion of the phenotype of Kosaki overgrowth syndrome, and description of the long-term outcome in the oldest case

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Introduction: Kosaki overgrowth syndrome (KOGS), first described in 2015, is a rare overgrowth disorder caused by *de novo* pathogenic variants in the *PDGFRB* gene. Only 5 paediatric cases have been reported in the literature.

Material and Methods: We present 3 cases of KOGS living in Europe including the first adult patient, gathered through a collaboration within the ERN ITHACA network.

Results: The French patient, aged 55, has in the past been reported as a typical case of Sphritzen-Goldberg syndrome (Stoll, Clin Dysmorphol 2012). Analysis of SKI was negative, and WES identified the recurrent *PDGFRB* c.1751C>G variant. The English patient, aged 6, diagnosed by NGS panel for syndromic overgrowth (de novo *PDGFRB* c.1751C>G variant), had tall stature, craniosynostosis, facial features, progressive contractures and developmental delay. The Norwegian patient, aged 9, diagnosed by WES (de novo *PDGFRB* c.1696T>C variant) had tall stature, severe scoliosis, facial features and developmental delay. Long-term outcome in the oldest patient revealed an evolving phenotype with persistent tall stature despite severe scoliosis not accessible to surgery (188 cm), extremely fragile skin with diffuse erythrosis, dystrophic scars due to multiple injuries, nail dystrophy, progressive camptodactyly, early osteoporosis, basilar artery aneurysm complicated by a stroke, and severe ocular impairment due

to complicated surgeries for pterygions. The patient had normal intelligence, with no cognitive decline nor cerebral calcifications.

Conclusion: Genotype-first approach is a powerful approach to reach the diagnosis of ultra-rare syndromes. Indeed, the clinical diagnosis was not raised before NGS, due to the lack of knowledge of this entity.

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P11.49A

Further delineation of the phenotypic spectrum of MCAP in a French cohort of 31 patients

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Introduction: MCAP syndrome (megalencephaly-capillary malformation syndrome) is a genetic disorder that results from somatic, mosaic, gain of function mutations of the *PI3KCA* gene, and belongs to the spectrum of PIK3CA-related overgrowth spectrum (PROS).

Material and Methods: Based on a national collaboration, we described the clinical features of 31 patients with MCAP syndrome carrying mosaic *PIK3CA* pathogenic variants. The objective of this study was to better define the clinical features of patients with MCAP syndrome in connection with imaging features, to improve diagnosis and management.

Results: The mosaic rate was between 0% and 20% in the blood, between 1% and 47% in the skin, and between 4% and 34% in the saliva. Macrocephaly had been observed in 21/28 patients (75%) at birth, and in 26/28 patients (93%) at time of the diagnosis. Hemihypertrophy was noticed in 22 patients (71%). Intellectual disability was present in 15 patients (50%), ranging from mild to severe, 4 patients had learning disabilities (12%). Six patients suffered from seizures (20%). Cerebral malformations were reported in 23/26 patients: ventriculomegaly (65%), megalencephaly (53%), cerebellar tonsilla ectopia (35%), polymicrogyria (15%). Correlations between neurogognitive development and cerebral malformations were made. All patients had cutaneous vascular malformation: angioma (53%), cutis marmorata (29%), capillary malformations (26%). Internal vascular anomalies are reported on 5 patients (16%). Limb and distal anomalies were also observed.

Conclusion: This study confirms the large clinical heterogeneity in MCAP, in particular in the neurocognitive development. This point is of importance for determining the outcome measures for future therapeutic trials.

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P11.50B

Investigating the Clinical Features Leading to Morbidity and Mortality in Microcephaly-Capillary Malformation Syndrome

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Microcephaly-capillary malformation syndrome (MIC-CAP) is a rare neurocutaneous disorder characterized by congenital microcephaly, multiple capillary malformations, hypoplastic distal phalanges, infantile-onset intractable seizures, profound neurologic impairment and abnormal neuroimaging findings including a diffusely simplified cortical gyral pattern with increased extra-axial space. It is caused by biallelic pathogenic variants in the *STAMBP* gene and has been reported in only 14 individuals worldwide, to date. Given the rarity, the full phenotypic spectrum remains poorly understood with limited natural history and prognosis information, as well as guidelines on optimal medical management. Additionally, there is one reported individual with a mild phenotype suggesting variable severity, with insufficient data to define genotype-phenotype correlations.

Here we present a case series of patients with MIC-CAP obtained through a prospective well-designed online survey of their primary caregivers and physicians. In this study, we describe the most frequently reported features, management and impact on disease course. We specifically focused on hallmark features leading to morbidity and decreased lifespan including seizures, neurologic impairment and respiratory insufficiency. We report the various therapies used by affected individuals and perceived benefits of these therapies including anti-epileptics medications. Additionally, we describe the spectrum of severity of other major features in previously reported individuals, including an additional seven who have not been reported in the literature to identify genotype-phenotype correlations.

Improved understanding of the hallmark features, including those leading to morbidity and mortality, in MIC-CAP syndrome will facilitate early diagnosis, inform management strategies and provide prognostic information.

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P11.51C

Dominant negative effect of ACTG2 mutations influence TGF-beta signaling in MMIHS

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Introduction: Megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) is a rare inherited disorder characterized by bladder hypocontractility, and intestinal obstruction. Mutations in five genes have been identified as its cause, but the majority of cases are caused by *de novo* heterozygous mutations in the γ-smooth muscle actin gene (*ACTG2*). *ACTG2* encodes for an actin isoform specifically expressed in the intestinal and urogenital tracts. Previous studies by us and others, have shown that mutated ACTG2 disrupt actin polymerization and result in reduced cellular contractility. However, the mechanisms underlying these effects are still unknown.

Materials and Methods: Wild-type and mutant ACTG2 proteins fused to a GFP and mCherry tag respectively, were stably expressed in U2OS cells and human intestinal smooth muscle cells. Transfected cells were analyzed by immunofluorescence, actin polymerization and cellular contractility assays. In Parallel, fibroblasts derived from two MMIHS patients carrying *ACTG2* mutations, and three controls have been used for trans-differentiation assays. Immunofluorescence, qRT-PCR, and a TGF-beta reporter assay were performed, to investigate the effect of *ACTG2* mutations on cellular differentiation.

Results: Actin polymerization, and cellular contractility were significantly impaired when the mutant ACTG2 protein is expressed, alone or in combination with the wild-type. Furthermore, TGF-beta is up-regulated in fibroblasts derived from MMIHS patients when compared to controls.

Conclusion: *ACTG2* mutations lead to a dysregulation of the TGF-beta pathway, and reduce cellular contractility by exerting a dominant negative effect in MMIHS.

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P11.52D
22q11.2 deletion as a genetic model for multimorbidity in young adults

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Background: Multimorbidity is increasing in younger adults but is understudied in the general population. 22q11.2 deletion syndrome (22q11.2DS) could act as a genetic model of multimorbidity in young to middle-aged adults.

Methods: Using Anatomic Therapeutic Classification (ATC) and setting 5 or more concurrent prescription medications as a proxy for multimorbidity, we compared data on 264 adults with 22q11.2DS (median age 27.8, range 17.3-68.3 years) to that for a community-based general population sample in Canada (n=25,287). We used logistic regression to examine possible predictors of multimorbidity in 22q11.2DS.

Results: Multimorbidity in 22q11.2DS in the 25-44 year age group (34.7%) was significantly more prevalent than in the general population, both for the same age group (2.9%, prevalence ratio, PR=11.9) and compared to those aged 45-64 years (16.4%, PR=2.1). Neuropsychiatric and endocrinological ATC medication classes predominated. Within 22q11.2DS, older age and psychotic illness, but not sex, major congenital heart disease or intellectual disability, were significant predictors of multimorbidity.

Conclusion: The results indicate that adults with 22q11.2DS have a significant burden of illness with levels of multimorbidity comparable to those of the general population several decades older. In younger adults with multimorbidity, certain disease patterns may also help identify genetic disorders in "big data".

A.S. Bassett: None. **S.L. Malecki:** None.

P11.53A
Transversal cohort analysis with data aggregation of singleton exome sequencing significantly increases the diagnostic yield for the diagnosis of fetuses with multiple congenital abnormalities

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Exome sequencing (ES) harbors a diagnostic yield in fetuses with multiple congenital abnormalities (MCA) (25%) higher than any other diagnostic tool in fetal pathology but lower than in live birth individuals with malformations (30-35%). Most fetuses remaining undiagnosed deprive couples of obtaining precise genetic counseling and molecular diagnosis in future pregnancies. This limited yield is explained by fetal wide phenotypic variability and difficulties to define precise phenotypes. Here, we aimed to assess the contribution of ES to diagnose MCA fetuses and also identify new genes. We performed singleton ES in 95 MCA fetuses undiagnosed after current investigations. Fetal DNA quantity/quality permitted ES in only 95/151 fetuses. The strategy combined usual individual ES analysis and an original cohort analysis based on bioinformatics scores and variant databases independently of fetal phenotype. We selected variants on the following criteria: truncating with a significant probability of loss-of-function intolerance (pLI=0.9-1), reported in denovo-db database, reported (likely) pathogenic in ClinVAR database, or with probably recessive mode of inheritance. Usual individual ES identified pathogenic or likely pathogenic variants in 21/95 fetuses (22%). The cohort analysis identified not only these 21 variants but also additional pathogenic or likely pathogenic variants in 3/95 fetuses (4%), increasing the diagnostic yield from 22% to 25%. 9 candidate genes were suspected in 8/95 fetuses (8%) remaining to be replicated. In conclusion, this original strategy increased the diagnostic yield in a fetal cohort with atypical or non-described phenotype and could so be applied to other heterogeneous cohorts with developmental abnormalities.

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P11.54B
New recurrent 2q12.2q12.3 microdeletion involving *ST6GAL2* gene in a boy with neurological phenotype

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Recurrent microdeletion and microduplication syndromes are among the most common causes of human neurodevelopmental and psychiatric disorders. These reciprocal genomic disorders often result from non-allelic homologous recombination (NAHR) between near-identical segmental duplications. Copy number changes mediated by segmental duplications may be either pathogenic or may exist as benign variants in the human population.

However, almost all pathogenic CNVs are characterized by a range of phenotypic outcomes (variable expressivity) and by unaffected family members who carry the same CNV (incomplete penetrance). Here we report a boy with mild intellectual disability, speech delay, and hypotonia presenting a 1.56 Mb interstitial deletion at 2q12.2-12.3 inherited from his healthy father. The microdeletion includes three OMIM genes, of which *ST6GAL2*, in our opinion, is the most interesting. This gene encodes a beta-galactoside alpha-2,6-sialyltransferase expressed in brain and its transcription could be potentially activated for specific neuronal functions. In the literature four cases have been reported with microdeletions/microduplications comprising *ST6GAL2* gene and associated with neurological diseases. In DECIPHER database seventeen individuals have been identified with deletions similar to that of our case and neurological disease as intellectual disability, delayed speech, autism. Ten patients inherited the deletion from an apparently unaffected parent. It is interesting to note that all these microdeletions are very similar in extension and are possibly mediated by NAHR.

In conclusion, we describe a new recurrent microdeletion 2q12.2-12.3 containing the *ST6GAL2* gene, which may be a significant and independent risk factor for neurological diseases acting in association with other factors to modify neurological phenotypes.

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P11.55C
Expanding the LZTR1-RASopathy association: Three new cases of Noonan Syndrome with LZTR1 mutations

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Since the first publication in 2014, LZTR1 missense gain of function variants have been associated with Noonan Syndrome (NS) in both dominant and recessive pattern, with the dominant variants clustering on the different Kelch motifs of the protein. Here we present 3 new index cases of Noonan syndrome with variants in LZTR1. Two of them have novel unreported variants. Case 1. A 12 year old boy, with prenatal polyhydramnios, unilateral ptosis, relative macrocephaly, short stature (p10), cryptorchidism and pulmonary valve stenosis. He also presented mild language delay. Genetic testing identified a heterozygous missense variant c.848G>A p.(Arg283Gln) located on the Kelch4 motif previously reported in another NS individual (Umeki et al. 2018). Case 2. A 42 year old man with dysmorphic features of NS, short stature (p3), cryptorchidism, bicuspid aortic valve and visual loss. Genetic testing identified a heterozygous missense new variant c.289C>T (.(Arg97Trp) in the Kelch1 motif. The same variant was detected in his 69 year old mother who shared dysmorphic features and short stature. Case 3. A 22 year old woman with neonatal diagnosis of hypertrophic cardiomyopathy in context of multiple angiomas. She also presented short stature (p3), pectus carinatum, scoliosis and dysmoprhic facial features resembling Noonan syndrome. She is heterozygous for the new variant c.905C>T p.(Ala302Glu) located on the Kelch 5 motif. These 3 new cases in patients with clinical diagnosis of NS and variants in LZTR1 further help to delineate the phenotype of NS patients, expanding the LZTR1-RASopathy association

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P11.56D

Phenotypic spectrum associated with SPECC1L pathogenic variants: new families and critical review of the nosology of Teebi, Opitz GBBB, and Baraitser-Winter syndromes

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The SPECC1L protein plays a role in adherens junctions involved in cell adhesion, actin cytoskeleton organization, microtubule stabilization, spindle organization and cytokinesis. It modulates PI3K-AKT signaling and controls cranial neural crest cell delamination during facial morphogenesis. *SPECC1L* causative variants were first identified in individuals with oblique facial clefts. Recently, causative variants in *SPECC1L* were reported in a pedigree reported in 1988 as atypical Opitz GBBB syndrome. Six families with *SPECC1L* variants have been reported thus far. We report here eight further pedigrees with *SPECC1L* variants, including a three-generation family, and a further individual of a previously published family. We discuss the nosology of Teebi and GBBB, and the syndromes related to *SPECC1L* variants. Although the phenotype of individuals with *SPECC1L* mutations shows overlap with Opitz

syndrome in its craniofacial anomalies, the canonical laryngeal malformations and male genital anomalies are not observed. Instead, individuals with *SPECCL1* variants have branchial fistulae, omphalocele, diaphragmatic hernias, and uterus didelphis. We also point to the clinical overlap of *SPECC1L* syndrome with mild Baraitser-Winter craniofrontofacial syndrome: they share similar dysmorphic features (wide, short nose with a large tip, cleft lip and palate, blepharoptosis, retrognathia, and craniosynostosis), although intellectual disability, neuronal migration defect, and muscular problems remain largely specific to Baraitser-Winter syndrome. In conclusion, we suggest that patients with pathogenic variants in *SPECC1L* should not be described as "dominant (or type 2) Opitz GBBB syndrome", and instead should be referred to as "*SPECC1L* syndrome" as both disorders show distinctive, non overlapping developmental anomalies beyond facial communalities.

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P11.57A
Integrated clinical-molecular diagnostic criteria for mosaic overgrowth syndromes

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Establishing robust clinical diagnostic criteria for mosaic disorders is difficult because of variation in the number of mutant cells amongst patients. Using Proteus syndrome as an example, a patient with a high level of mutant cells is easy to diagnose, but one with few mutant cells may be impossible to recognize. Also, some tumors have the *AKT1* c.49G>A p.(E17K) variant, and this is not Proteus syndrome. Also, some aspects of the phenotype are not unique to Proteus syndrome. We therefore recognize that proper diagnosis must integrate both clinical and molecular aspects. To address this, we have developed an integrated, clinical-molecular diagnostic system that defines a robust threshold for Proteus syndrome *stricto sensu* and a broader category of what we term '*AKT1*-related overgrowth spectrum'. Each Proteus syndrome manifestation is assigned a weighted score with associated manifestations receiving a positive, weighted score (e.g., the cerebriform connective tissue nevus) and each atypical manifestation receiving a negative, weighted score (e.g., congenital, non-CNS overgrowth). The presence of an *AKT1* mosaic variant was assigned a high positive score. We piloted these criteria on the NIH mosaic overgrowth cohort and adjusted and adapted them to achieve agreement on appropriate thresholds. We suggest that this semi-quantitative, integrated clinical-molecular diagnostic approach is generalizable to other mosaic disorders, with its most important contribution being the formal recognition that patients with lower variant burdens have a related, but distinct and milder disorder that must be differentiated from the classic form of the disorder.

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P11.58B
Multiple anomalies in an adult case with pentasomy X

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Pentasomy X is a very rare chromosomal disorder, the exact prevalence is not known. Even rarer are descriptions of adult cases. We present a 20 years old girl hospitalized for functional rehabilitation. She was born from healthy, young, unrelated parents. Mother reported severe nausea and vomiting during early pregnancy, birth at term, pelvic presentation, Apgar 10. Medical history revealed multiple corrective orthopedic surgeries such as osteotomies and elongations/tendon transpositions for the skeletal abnormalities, operated and recidivate varus equin, bilateral genu valgum operated and relapsed, atrial septal defect without indication for surgery, moderate mental retardation, primary nocturnal enuresis, mixt astigmatism. She was diagnosed with cerebral palsy, spastic diparesis, periventricular leukomalacia. Clinical and functional evaluation revealed normal height, 174 cm, overweight, microcephaly, hypertelorism, epicantus, bilateral corneal leukoma, divergent strabismus, low-set ears and mandibular prognathism. She had many bone and joint anomalies such as clinodactily, right varus equin, bilateral genu valgum with marked latero-lateral and antero-posterior instability, congenital right hip dislocation with femoral head necrosis, congenital left hip subluxation, shortening of the right lower limb by 2 cm, right radial head subluxation with functional impairment of

the right elbow, hyperextension of the left elbow, moderate motor deficit and muscular hypotonia in both lower limbs, dorso-lumbar sinistro-convex scoliosis, dorsal kyphosis, lumbar lordosis, generalized ligamentous laxity. She was able to walk independently with right knee marked deviation in valgus, typical cerebral palsy gait. Our patient was a rare case, an adult with atypical height, multiple musculoskeletal congenital defects associated with eye and heart anomalies.

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P11.59C
First case of osteolysis in a patient with clinical and molecular diagnosis of CLOVES syndrome

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The *PIK3CA* Related Overgrowth Spectrum (PROS) includes a large number of pathologies showing clinical overlap due to sharing the same causing gene (*PIK3CA*) and genetic mechanism (somatic mosaicism). The clinical manifestations in PROS depend on the time and place during the embryonic development in which the mutation occurred. One of the most recognizable syndromes within the PROS spectrum is CLOVES (Congenital Lipomatous Overgrowth, Vascular malformations, Epidermal nevi, Scoliosis/Skeletal/Spinal anomalies), which presents a larger clinical severity associated with a wide distribution of affected tissues due to the appearance of early mutations. Recently, we have described that Generalized Lymphatic Anomaly (GLA) may also be caused by somatic, activating mutations in *PIK3CA*. An intriguing clinical feature in GLA is the presence of osteolysis associated with hyperplastic lymphatics in bone. It is not known why bone loss occurs in GLA but has not been described in isolated lymphatic malformations or in the rest of the PROS syndromes with more pleiotropic clinical features. We present a patient with a clinical diagnosis of CLOVES, in whom there is also a vertebral lytic lesion accompanied by a venous/lymphatic component. The patient shows the variant *PIK3CA:* c.241G>A;p.Glu81Lys, detected in a mosaic of 8.53% in the affected tissue and absent in blood and saliva samples. This is the first description of phenotypic overlap between osteolysis and other PROS spectrum syndromes, supporting the inclusion of GLA within this spectrum. This research was supported by the project IP-17 from the call "Todos Somos Raros", and Co-financed by ISCIII, FEDER FUNDS FIS PI17/00519.

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P11.60D
Mosaic activating *PIK3CA* mutations during early embryonic development cause ectopic muscle development and upper limb overgrowth

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Activating somatic mutations in the phosphatidylinositol-4,5-bisphospate 3-kinase, catalytic subunit alpha (*PIK3CA*) gene cause *PIK3CA*-Related Overgrowth Spectrum (PROS). The timing and location of the *PIK3CA* mutations can explain the phenotypic heterogeneity within PROS. Here, we present a 4-year-old male individual (P1) and an 18-year-old female individual (P2) with a PROS phenotype with isolated muscle overgrowth of upper limbs and ectopic muscles. P1 had four ectopic muscles and unilateral isolated muscle overgrowth and P2 had 13 ectopic muscles and bilateral symmetric isolated muscle overgrowth of upper limbs. Sequencing and digital PCR on DNA extracted from biopsies from hypertrophic ectopic muscles identified mosaic *PIK3CA* mutations p.His1047Arg (VAF 18%) and p.Glu542Lys (VAF 26%) in P1 and P2, respectively. Since P2 has bilateral findings, the mutation might have occurred before day 15 after fertilization in a cell of the primitive

streak in the middle of the embryo. The mutation in P1 might have occurred after day 21 post-fertilization since his muscular overgrowth is unilateral. Observation of supernumerary muscles in locations in the upper extremity where there should not be muscles implies that the cells with *PIK3CA* mutations might have differentiated into muscles instead of tendons or fascia. Our findings highlight that the timing of *PIK3CA* mutagenesis during embryonic development has an impact on the phenotype and PI3K has a role in pluripotency and cell fate in early human development.

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P11.61A
Clinical and genetic features of prader-willi syndrome in vietnam

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Introduction: Prader-Willi syndrome (PWS) is a complex genetic disorder that results from the lack of paternally expressed in the chromosome 15q11-q13 region. This study was performed to delineate the clinical and genetic features of children with PWS.

Methods: The clinical signs were studied. Karyotype, fluorescence in situ hybridization, methylation- specific polymerase chain reaction and subsequent methylation-specific multiplex ligation- dependent probe amplification were used to describe characteristics of genetic classes of PWS. Data were collected on 101 patients with a molecular diagnosis of PWS at Vietnam National Children's Hospital.

Results: The mean age at diagnosis was 30.2 months. Most patients who showed the characteristics of facial dysmorphism. 92.1% had neonatal hypotonia and feeding difficulties, 61.4% of the neonates were hospitalized, 11.8% had obesity, 79.6% showed developmental delays and cryptorchidism was 84%. Among 101 patients, 51 patients had been treated with growth hormon (GH) and GH treatment resulted in an increase of insulin-like growth factor 1 (IGF-1) and a decrease in obesity. Deletion subtype was present in 86 patients (85.1%), 4 patients had a translocation, one case is an unique PWS deletions. mUPD, ID were observed in 15 patients (14.9%). Significantly older mothers (mean age= 31.9 years vs 28.2 years) were found in the mUPD, ID group compared with the deletion subtype.

Conclusions: The mean age at diagnosis was late. Increase IGF-1 and decrease obesity rate after GH treatment. The risk of mUPD may also increase with maternal age or this suggests that ethnic differences be relevant for PWS.

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P11.62B
A lower BMI and growth hormone use results in decreased mortality in Prader-Willi syndrome

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Early mortality has been reported in PWS. Recent studies have shown an increase in survival estimates in the last 20 years. The hypothesis is that it is due to preventative measures to avoid morbid obesity. The PWS Association (PWSA) USA created a long standing bereavement and research program to investigate causes of death and collect data in living individuals with PWS. A familial-response questionnaires from the PWSA (USA), tested the hypothesis that body mass index (BMI), age of diagnosis, clinical symptoms, and growth hormone treatment differ among deceased and living individuals with PWS. Data were available on a total of 2,029 individuals with PWS (114 deceased and 1,915 living) from the USA. Categorical and continuous variables were compared using chi-square and two-group t-tests, respectively. For categorical variables, the effect of age was limited by stratifying for age (<20y v. ≥20y) and birth year (<1994 v. ≥ 1994) and testing with a Mantel-Haenszel test. For continuous variables, the effect of age was limited by adjusting for age as a continuous

variable in logistic regression. Average age at death was 31.6 years. Deceased individuals had lower rates of growth hormone use ($p<0.001$) and higher rates of increased weight compared to living individuals. BMI in living and deceased individuals with PWS were 28.6 (SD=11.9) and 51.7 (SD=21.7), respectively ($p<0.001$). This study highlights the benefits of growth hormone, external control of weight, early diagnosis, and the need for a low threshold for bringing affected individuals to medical attention.

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P11.63C

Neutralization of heat shock transcription factor 1 in cells from segmental overgrowth syndrome patients blocks abnormal proliferation

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PIK3CA-Related Overgrowth Syndromes (PROS) are caused by mosaicism mutations in the PIK3CA gene. The use of specific inhibitors to target PI3K or mTOR in PROS was recently found to lead to disease regression. However, the downstream effectors of the PI3K/mTOR pathways specifically involved in the pathogenicity of PROS remain unknown. Heat shock transcription factor 1 (HSF1) is the major stress-responsive transcription factor. Recent findings indicate that AKT phosphorylates and activates HSF1 independently of heat-shock in breast cancer cells. We aimed to investigate the role of HSF1 in PROS.

We found elevated phosphorylation and activation of HSF1 in cells from PROS patients, which are directly correlated to the presence of activated form of AKT. In line with its function as a transcription factor, active HSF1 localized in the nucleus of cells from PROS but not in control donors. Inhibition of PI3K or mTOR activity strongly reduced HSF1 activation in patient cells. Messenger RNA expression of 47 genes that are known to be regulated by HSF1 were repeatedly down-expressed upon PI3K inhibition. We also observed that targeting HSF1 with specific inhibitors reduced the proliferation of mutant cells as efficiently as PI3K or mTOR inhibitors. The increased number of cells entering the S phase of the cell cycle, which is characteristic of PROS, was completely blunted by HSF1 inhibitors. Interestingly, CCND2, a major cell cycle regulatory protein was down-expressed upon functional inhibition of HSF1.

Conclusion: Our results identify HSF1 as a new potential therapeutic target in PROS.

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P11.64D

PUF60 splicing variant in a boy with coloboma, short stature and subtle facial features

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Recent studies have clarified that microdeletion involving *PUF60* [Poly(U) Binding Splicing Factor 60] and de novo variants in *PUF60* cause multiple congenital anomalies and intellectual disability. We report a boy with a *PUF60* variant and coloboma, congenital heart disease, and short stature. He was born uneventfully to healthy unrelated parents, with around average birth weight and height. He underwent surgical ligation at 7 months for symptomatic patent ductus arteriosus. He had recurrent febrile seizures in infancy. He walked by 17 months and spoke his first word by 15 months. His facial features include a square face, webbed neck, broad nasal bridge, long eyelashes, dark thick eyebrows, flat philtrum, thin upper vermilion and dental crowding. His square face and large teeth were similar to KBG syndrome. He had a right choroidal coloboma (corrected visual acuity 0.06), short stature (Z-score -2.5) and borderline head circumference. He goes to junior high school without special needs. Whole exome sequencing of the boy and his parents identified a de novo novel variant in *PUF60* [NM_078480, c.297+1G>A]. RT-PCR analysis of lymphoblastoid cells from the patient and a control identified an abnormal splicing event involving intron 4 sequences in the patient but not in the control. The abnormal splicing product underwent nonsense mediated mRNA decay.

Conclusion: This case of a *PUF60* pathogenic variant in a boy with coloboma, congenital heart disease, short stature, subtle facial features, and normal intellectual development suggests that *PUF60* variants should be considered in

syndromic phenotypes with coloboma with or without intellectual disability.

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P11.65A

Novel homozygous nonsense *PXDN* mutation detected by whole exome sequencing causing anterior segment dysgenesis

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Anterior segment dysgeneses (ASMD - MIM #269400) encompass a spectrum of anterior chamber ocular developmental defects affecting the cornea, iris, and lens and are generally associated with a significant risk for glaucoma. Clinical features include iris hypoplasia, corneal opacity, posterior embryotoxon, corectopia, polycoria, an abnormal iridocorneal angle, ectopia lentis, and anterior synechiae between the iris and posterior corneal surface.

We report on a Greek-Cypriot male born to parents who were distantly related. He presented with bilateral iris defects, extensive anterior synechiae, posterior embryotoxon, nystagmus, glaucoma and cataracts. Other non-ocular abnormalities included bilateral hydronephrosis, vesicoureteric obstruction, bilateral megaureter and bipolar disorder.

Negative investigations included array-CGH, MLPA for *PAX6* deletions and Sanger sequencing of *PAX6, FOXC1* and *PITX2*. Trio-based WES was subsequently performed on the Illumina NextSeq500 platform using the TruSeq DNA exome library kit. Bioinformatics analysis was carried out using an in-house pipeline according to the Genome Analysis Toolkit Best Practices.

Homozygosity for a novel nonsense *PXDN* p.(Gly700*) variant was identified in the index case. The variant was confirmed by Sanger sequencing as was parental carrier status.

In conclusion, a novel, homozygous *PXDN* variant was identified with a strong phenotypic correlation. Other reports of *PXDN* mutations have been associated with autosomal recessive ASMD in patients with similar phenotypes. It is currently unknown whether the proband's non-ocular phenotypes are linked to *PXDN*; therefore, more similar cases are needed to reach a correlation. These results highlight the strength of WES as a diagnostic tool and the diversity of phenotypes caused by *PXDN* mutations.

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P11.66B

A clinical utility study of diagnosing genetic disorders in newborns admitted to the Neonatal Intensive Care Unit

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Rapid whole exome sequencing (rWES) is feasible, but awaits embedding in routine clinical care in most hospitals. We hypothesized that routine rWES will profoundly increase genetic diagnoses for critically ill newborns, leading to improved patient care. Therefore we performed a clinical utility study assessing the value of genetic testing in routine neonatal clinical practice. In a multi-centre prospective study set-up, 60 critically ill newborns with a presumed genetic disorder received rWES, parallel to routine genetic testing. To obtain insight into the prevalence of genetic testing in the first 30 days of life, historical data from all 1423 patients admitted to the NICU in the Radboudumc (October 2013 to October 2015) served as a control period.Prospectively, a conclusive molecular diagnosis was obtained in 19 of 60 patients (31.7%), with a median time-to-diagnosis of 14 days (IQR 11-21). In comparison, genetic testing was performed in 149 patients (10.5%) in the control period, leading to a conclusive molecular diagnosis within 30 days of life in 13 of them (8.7%). The median time-to-diagnosis was 14 days (IQR 12-15). Importantly, from our control period, we noticed that in a further 136 patients (9.6%) genetic test reports outlasted the first 30 days of life, but increased the diagnostic yield to 32.2%. This suggests that more NICU patients may benefit from rWES than currently tested. Our data show that the introduction of rWES for all critically ill newborns with a presumed genetic disorder increases diagnostic yield earlier in life than conventional diagnostic tests.

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P11.67C

Is Schilbach-Rott/Blepharofacioskeletal Syndrome caused by a mutation in *HNRNPH1?* - a report on a 2 cases

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Schilbach-Rott/Blepharofacioskeletal Syndrome (SR/BRSS) (OMIM #164220) is an autosomal dominant condition characterized by blepharophimosis, cleft palate, dysmorphic facies, and hypospadias in males. In many patients hypotelorism and mild skeletal anomalies in the hands or feet have been reported. No causative gene of SR/BRSS has been found to date. We present the clinical characteristics of two boys with severe developmental delay and dysmorphic features suggestive of SR/BRSS. In both, blepharophimosis, cleft or high-arched palate, hypospadias, scoliosis, and arachnodactyly with clinodactyly were observed. Additionally microcephaly, arched eyebrow, high narrow nasal bridge, long hanging columella, micrognathia, hypoplastic earlobes, hypermobile joints have been noted. The same novel *de novo* mutation, *HNRNPH1* c.616C>T (p.Arg206Trp), was found in both patients using whole exome sequencing. To date, mutations in the highly conserved autosomal *HNRNPH1* gene have not been associated with human disease. HNRNPH1 belongs to the group of nuclear ribonucleoproteins involved in regulation of pre-mRNAs splicing. Interestingly, the identical recurrent mutation affecting the small domain encoding nuclear localization signal in the highly paralogous *HNRNPH2* gene has been previously associated with X-linked, syndromic, Bain type mental retardation [MRXSB, OMIM #300986]. MRXSB has been identified in females with developmental delay, intellectual disability, autism, hypotonia, and seizures. Only in single cases hypotelorism, small palpebral fissures, or elongated fingers were observed. Although defective function of HNRNPH2 and HNRNPH1 proteins have similar clinical consequences in regards to intellectual disability, mutations in *HNRNPH1* gene may represent a distinct syndrome perhaps related or identical to Schilbach-Rott/Blepharofacioskeletal Syndrome. Grant Numbers: KNW-1-036/K/7/K; 2013/11/B/NZ7/04944;

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P11.68D

SEC31A mutation affects ER homeostasis, causing neurological syndrome

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Introduction: Consanguineous kindred presented with an autosomal recessive syndrome of intra-uterine growth retardation, marked developmental delay, spastic quadriplegia with profound contractures, pseudobulbar palsy, epilepsy, dysmorphism, neurosensory deafness and optic nerve atrophy with no eye fixation. Affected individuals died by the age of four. Brain MRI demonstrated microcephaly, semilobar holoprosencephally and agenesis of corpus callosum. We aimed at elucidating the molecular basis of this disease.

Methods: Genome-wide linkage analysis combined with whole exome sequencing were performed to identify disease-causing variants. Functional consequences were investigated in fruit flies null mutant for the Drosophila *SEC31A* orthologue. *SEC31A* knockout SH-SY5Y and HEK293T cell-lines were generated using CRISPR/Cas9 and studied through qRT-PCR, immunoblotting and viability assays.

Results: Through genetic studies, we identified a disease-associated homozygous nonsense mutation in *SEC31A*. We demonstrate that *SEC31A* is ubiquitously expressed, and that the mutation triggers nonsense mediated decay of its transcript, comprising a practical null mutation. Similar to the human disease phenotype, knockdown *SEC31A* flies had defective brains and early lethality. Moreover, in line with *SEC31A* encoding one of the two coating layers comprising the COP-II complex, trafficking newly synthesized proteins from the endoplasmic reticulum to the Golgi, CRISPR/Cas9-mediated *SEC31A* null mutant cells demonstrated reduced viability through upregulation of ER-stress pathways.

Conclusions: We demonstrate that a severe neurological syndrome is caused by a null mutation in SEC31A, reducing cell viability through enhanced ER-stress response, in line with SEC31A's role in the COP-II complex. Funding: Legacy Heritage Bio-Medical Program of the Israel Science Foundation (grants no. 1814/13 and 1798/16)

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P11.70B

SMCHD1 mutation spectrum for facioscapulohumeral muscular dystrophy type 2 (FSHD2) and Bosma arhinia microphthalmia syndrome (BAMS) reveals disease-specific localization of variants in the ATPase domain

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Introduction: Variants in the chromatin modifier Structural Maintenance Of Chromosomes Flexible Hinge Domain-Containing Protein 1 (SMCHD1) have been associated with the myopathy FSHD2 and with the unrelated nasal developmental syndrome BAMS. FSHD2 is a digenic hereditary disease with heterozygous variants anywhere in SMCHD1 causing toxic derepression of *DUX4*, a gene that is embedded in the D4Z4 repeat on chromosome 4 variant 4A. BAMS also requires missense variants in SMCHD1, but specifically in the ATPase domain. Irrespective of the phenotypic outcome, both FSHD2 and BAMS associated SMCHD1 variants result in quantifiable hypomethylation of the D4Z4 locus. We identified numerous new pathogenic FSHD2 variants and non-pathogenic variants and compared these to BAMS associated variants. M&M: Genetic and methylation studies of D4Z4 and SMCHD1 in 91 new FSHD2 families. Examination of the position and mutation type for SMCHD1 variants in 180 (89 from previous publications) FSHD2 families, 41 BAMS patients and 57 control individuals. Generation of a 3D model for the SMCHD1 ATPase-domain in which we modeled all missense variants in FSHD2 and BAMS.

Results: The FSHD2 mutation spectrum shows missense, nonsense, indels and splice site variants covering the entire SMCHD1 locus, but missense variants are specifically enriched in the ATPase domain. Non-pathogenic missense

variants were almost absent from the ATPase domain. FSHD2 and BAMS variants occupy different sites in the 3D model of the ATPase domain.

Conclusions: The localization within the ATPase-domain might underlie the phenotypic outcome of missense variants in the ATPase-domain of SMCHD1. Funded by NIH and Prinses Beatrix Spierfonds.

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P11.71C

SMG9 deficiency syndrome: confirmation and further phenotypic characterization

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Introduction: SMG9 deficiency syndrome is a rare autosomal recessive condition originally described in three patients from two families with homozygous truncating *SMG9* variants. Affected individuals presented with a severe syndromic developmental disorder, and a mouse model revealed the embryonic lethality of bi-allelic *Smg9* deficiency, in a context of major brain, eye and cardiovascular malformations. To our knowledge, no additional patient has been described since this report.

Methods: We performed whole exome sequencing in a patient exhibiting a syndromic developmental delay and in her unaffected parents and report the phenotypic features.

Results: Our patient was born to consanguineous parents at 28 weeks of gestation. During pregnancy follow-up, left cleft lip and alveolus, polyhydramnios and intrauterine growth restriction were noticed. Postnatal examination revealed prominent forehead, hypertelorism, ears abnormalities, cataracts, kyphosis, renal hypoplasia, as well as complex ventricular septal defects and aortic bicuspid valve on cardiac ultrasound and thin corpus callosum and dilated ventricles on brain MRI at the age of 12 months. She presented early and severe feeding difficulties, severe growth restriction, global developmental delay, and multiple severe infections requiring hospitalizations. She carried a novel c.1177C>T, p.(Gln393*) (NM_019108) *SMG9* homozygous variant while her unaffected parents were both heterozygous.

Conclusions: We confirm that bi-allelic truncating *SMG9* variants cause a severe brain and heart malformation syndrome including ventricular septal defects, brain abnormalities, facial dysmorphic features, and severe growth and developmental delay (4/4) with or without ophthalmological abnormalities (3/4), severe feeding difficulties (2/3), and life-threatening infections (2/4).

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P11.72D

The complete loss of function of the *SMS* gene is responsible for an antenatal onset and a lethal form of Snyder-Robinson Syndrome

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Introduction: Snyder-Robinson syndrome (SRS) is an X-linked syndromic intellectual disability condition with postnatal expression caused by variants in the *Spermine Synthase* gene (*SMS*). The syndrome is characterized by facial dysmorphism, thin habitus, hypotonia and a

nonspecific movement disorder. Until now, only missense variants with a functionally characterized partial loss of function (LoF) have been described. Here we report the first complete LoF variant in a male patient with a congenital form of SRS. Patient and Methods: The patient had multiple malformations with antenatal onset and died at 4 months of age after staying in the intensive care unit. His phenotype included IUGR, cysts of the vermis, facial dysmorphism, hypotonia with no eye contact, complex cardiac, gut and kidney malformations. His DNA and that of his parents were sent to our genetics laboratory to perform whole-exome-sequencing. Functional studies have been performed to validate a variant found by WES: western blot analysis to quantitatively determine the protein level of SMS and UPLC - MS/MS to qualitatively determine the enzyme activity.

Results: Genomic analysis revealed a *de novo* hemizygous frameshift in *SMS*, c.906_909delAATG; p.M303Kfs3, classified as pathogenic according to the ACMG criteria. Functional *in vitro* assays showed a complete absence of functional SMS protein.

Conclusion: Taken together, our findings confirm that the mechanism of pathogenicity in SRS is a LoF in *SMS* and that complete LoF leads to a far more severe phenotype than partial LoF due to missense variants.

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P11.73A

Rare case of a complex small supernumerary r(1;10) chromosome mosaicism associated with a Kabuki-like phenotype

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Introduction: Complex small supernumerary marker chromosomes (sSMC) originate from more than one chromosome and can cause variable phenotypes. We report the first case of mosaic ring-shaped sSMC derived from chromosomes 1 and 10 in a child with dysmorphisms reminiscent of Kabuki Syndrome (KS). KS is a genetically heterogeneous autosomal dominant condition characterized by intellectual disability (ID), multiple congenital anomalies and a recognizable facial gestalt. Case presentation: The 3-year-old girl showed moderate ID, aggressive behaviour and dysmorphisms, including hypertelorism, long palpebral fissures, eyebrows with sparse lateral third, and short columella, resembling KS patients. ArrayCGH identified a 40.76-Mb pathogenic duplication at 1p13.3q21.2 pericentromeric region and a 4.69-Mb duplication of unclear clinical significance at 10q22.2q22.3, both with profiles suggesting mosaicism. Subsequent karyotype and FISH analyses revealed a supernumerary ring chromosome in 40% of the cells that co-hybridizes with probes for both chromosome 1 and 10 duplicated regions.

Discussion: sSMC derived from chromosome 1 can present a variable phenotype, ranging from normality to severe intellectual disability. However, the region 1p12 to 1q12 appears to be non-dosage dependant and the size of the sSMC seems to correlate with the severity of the phenotype. The resemblance with KS was interestingly reported 20 years ago in a patient presenting an interstitial duplication of the short arm of chromosome 1, in a *locus* with significant overlap with ours. In conclusion, we illustrate a distinctive phenotype of a rare chromosome abnormality, while emphasizing the importance of resorting to complementary classical cytogenetic studies.

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P11.74B

Tetrasomy 21 pter→q21.3 due to an extra +dic(21;21) in a severe psychomotor retarded female without Down syndrome phenotype

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Introduction: Complete or partial tetrasomy 21 without mosaicism has been reported in only rare cases. We report a Japanese female patient with tetrasomy 21 due to an extra chromosome derived from chromosome 21.

Materials and Methods: Chromosome, fluorescence in situ hybridization (FISH) and whole genome sequencing (WGS) analyses for the patient, and SNP array-based genotyping for the trio were carried out.

Results: The patient had severe psychomotor retardation without Down syndrome (DS) phenotype; she showed short stature, microcephaly, round face and some dysmorphic features. At eight years old, she could not speak any meaningful words nor walk. The developmental quotients score was below 20. The chromosome analyses detected an extra dicentric chromosome 21, and revealed her karyotype

to be 47,XX,+dic(21;21). FISH results were consistent with the karyotype. Allelic ratios of heterozygous SNPs indicated the maternal origin of the extra chromosome 21. Copy number and structural variant analyses using WGS data indicated that the distal breakpoint of the dicentric chromosome 21 is located within 21q21, and that the extra chromosome 21 doesn't simply consist of inverted duplications of pter→q21.3region but contain multiple partial deletions, duplications, and inversions within it.

Conclusions: The patient' lack of DS phenotype turned out to be due to the normal copy number of DS critical region (21q22.2-22.3). We will present the genomic organization of the tetrasomic region being further assessed by FISH and WGS data, and discuss possible molecular mechanisms that lead to the complex genomic rearrangements in the patient.

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P11.75C

Delineating the clinical spectrum due to heterozygous TRAF7 missense mutations: A series of 38 cases

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TRAF7 belongs to the Tumor necrosis factor receptor-associated factor (TRAF) family, involved in a broad range of biological functions and recently identified by WES in 7 patients associating developmental delay, congenital malformations and dysmorphic features with *de novo* missense mutations.

We gathered a series of 38 patients (a family with 3 cases and 35 sporadic cases) by WES, direct sequencing and GeneMatcher with heterozygous missense mutations within the WD40 repeats, 8 being recurrent. Facial features are distinctive and will be described. Many are short (12) with a short neck (20), pectus carinatum (14) and anomalies of the extremities with camptodactyly (8), ulna or radial deviation of fingers (10) and overriding toes (9). Ribs and vertebrae anomalies are each described in 5 and 16 cases. Conductive and/or sensorineural hearing loss is frequent (20). Congenital cardiac defects are also frequent with persistent ductus arteriosus (21) and atrial septal defects (9) being the most frequent. Worth noting are kidney abnormalities (10), genitalia anomalies (4), umbilical and/or inguinal hernia (8), dental anomalies (9), sparse hair (5) and lymphedema of lower limbs (3). Most patients presented feeding difficulties and motor delay with independent walking between 18 and 24 months of age. They developed mild to moderate

intellectual deficiency with speech delay, frequent nasal speech and usually preserved social interactions.

A different repertoire of somatic missense mutations within the WD40 domains of TRAF7 has been identified in meningioma. The clustering of germline and somatic missense mutations suggests a gain of function or a dominant negative effect.

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P11.76D
Biallelic variants in *TRAPPC11* cause syndromic intellectual disability

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TRAPPC11 is a component of the TRAPP complex involved in intracellular vesicle trafficking. *TRAPPC11* mutations were reported in only 20 patients with various phenotypes from limb-girdle muscular dystrophy (LGMD) phenotype to congenital disorder of glycosylation (CDG), characterized by frequent proximal muscle weakness with elevated creatine kinase (CK), associated with global developmental delay, and movement disorders. Using trio whole exome sequencing (WES), we identified two new patients with intellectual disability (ID) carrying biallelic variants in *TRAPPC11*. Patient 1: Pregnancy was marked by agenesis of corpus callosum (ACC) and intrauterine growth retardation (IUGR). At 4 years old, he presented neurodevelopemental delay, progressive microcephaly (-4SD) and small stature (-2.5SD). Immune screening showed hypogammaglobulinemia, causing multiple infections. CK level was normal. Postnatal brain MRI showed global cerebral atrophy and thin corpus callosum. He carried compound heterozygous splicing variants c.965+5G>T/c.1287+5G>A in *TRAPPC11* inherited from both parents. Patient 2: Pregnancy was marked by IUGR. He had epilepsy with onset at 5 months of age. At 30 months old, he presented with hypotonia, neurodevelopemental delay, and progressive microcephaly (-5SD). He had mildly elevated CK without myopathy. Brain MRI showed thin corpus callosum. He carried homozygous variants c.1287+5>A in *TRAPPC11*, both inherited from his father, due to uniparental isodisomy (UPD) of chromosome 4. We confirm the extended phenotype related to *TRAPPC11* variants, including syndromic ID. Hypogammaglobulinemia was not reported as related to this gene so far. Moreover, this report demonstrates the importance to explore UPD in case of homozygous variants inherited from a single parent.

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P11.77A
First mutation in *TSHZ1* in a family with Rasmussen syndrome

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In 1979, Rasmussen reported of a family with congenital bilateral vertical talus, bilateral external auditory canal atresia and increased interocular distance. After Rasmussen syndrome was mentioned in 2002 for the first time, Feenstra *et al*. reported of families with deletion or mutations in *TSHZ1* causing congenital aural atresia (CAA), in 2011. Here, we describe for the first time a family with vertical talus (VT) and mutation only affecting *TSHZ1*.

We report on a family with an eleven year old boy, who was born at term after uneventful pregnancy. The boy had CAA and VT. He had mild intellectual disability and facial features including epicanthus, hypertelorism and broad nasal root. The mother of non-consanguineous parents had learning difficulties as well as CAA and VT. The grandfather had CAA like several members of the family of the mother, too. In addition, the daughter of one cousin had CAA and VT.

In suspicion of Rasmussen syndrome a targeted sequencing analysis of *TSHZ1* identified a heterozygous frameshift mutation c.718delA;p.Arg240Gly*fs**10 in the index patient and his mother resulting in a premature stop of translation.

Systematic review of the literature showed that affected persons of two families with mutations in *TSHZ1* show CAA but VT has not been described in these patients. Therefore, we show for the first time the combination of VT and CAA due to mutation in *TSHZ1*.

Therefore, our findings support the hypothesis that heterozygous mutations in *TSHZ1* cause Rasmussen syndrome with incomplete penetrance and variable clinical expressivity.

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P11.78B
Mutations in TUBB4B can cause a cliopathy

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Introduction: Recently, we reported heterozygous mutations affecting Arg391 in the β-tubulin 4B isotype-encoding gene (TUBB4B) dampening the dynamics of growing microtubules (MT) in a distinctive neurosensorineural disorder (early-onset and severe retinal dystrophy and sensorineural hearing loss). Despite abnormal MT dynamics, patient fibroblasts and cells overexpressing the mutant proteins displayed apparently normal ciliation and trafficking. Here, we report the identification of a novel TUBB4B mutation in a sporadic case displaying blindness, sensorineural hearing loss, short stature, chronic kidney disease and recurrent sinus and ear infections, a constellation of symptoms strongly reminiscent of ciliopathies. Consistent with this phenotype, we show that the mutation which affects Pro358 have a dramatic effect on ciliation.

Material and Methods: Cilia from nasal brushing of the index cases were analyzed by TEM. The dynamics of MT growth and ciliation were analyzed in cultured cells overexpressing FLAG-tagged wild-type or mutant *TUBB4B*.

Results: Cilia from nasal brushing of the index case revealed ultrastructural abnormalities with rare axonemes and disorganized basal bodies. Inspection of the β-tubulin atomic structure revealed that the Pro358 residue is located in a binding domain necessary for the dynamics of microtubules. Functional analysis in cultured cells overexpressing FLAG-tagged wild-type or mutant TUBB4B showed that the mutant TUBB4B have a drastic impact on microtubule growth with no lattice as well as on ciliogenesis with absent cilia in the vast majority of cells.

Conclusion: This study shows that *TUBB4B* mutations can cause a sensorineural disease or a multisystemic ciliopathy, depending on the localization of the alteration.

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P11.79C
Further delineation of O2HE syndrome due to *UNC45A* biallelic variants and launching of the initiative adopt a gene for *UNC45A*

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Introduction: Only 4 patients with *UNC45A*-related osteo-oto-hepato-enteric syndrome (O2HE) have been reported in 2018, and no further replication has been published. The four core clinical features include congenital diarrhea, cholestasis, bone fragility, deafness, associated to learning disability.

Material and Methods: We report two novel cases of O2HE diagnosed by trio WES, enlarging the clinical spectrum by the presence of malformations.

Results: In patient 1 (20 years), severe congenital diarrhea due to microvilli atrophy was diagnosed at week 1, requiring long-term hospitalization with exclusive enteral nutrition. Diarrhea became intermittent until age 10 and then stopped. Chronic pruritus caused by congenital cholestasis was diagnosed in childhood. He was blind due to right anophthalmia, Peters anomaly with left pyramidal cataract, had severe sensorineural deafness, severe intellectual disability with behavioral disorder, had 2 fractures after minimal injury. Trio WES revealed a paternally inherited *UNC45A* c.1407delinsGCA variant and a maternally inherited c.2467G>C variant; and a *de novo PBRM1* VUS (c.1301G>A). In patient 2 (3 weeks), cleft palate and severe diarrhea was diagnosed at day 1, with flattening of brush border, requiring total parenteral nutrition. No cholestasis was diagnosed, and testing of the audition is planned. Rapid trio exome revealed a maternally inherited c.721C>T;p.(Arg241*) *UNC45A* variant and paternally inherited c.2182G>A variant.

Conclusion: Further cases will be needed in order to better describe the phenotypic spectrum of *UNC45A*, and particularly the level of the neurocognitive phenotype, and the associated congenital malformation spectrum. Therefore, we decided to adopt this gene, to compile the clinical consequences of novel variants.

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P11.80D
The first 30 months of experience of the Telethon Undiagnosed Diseases Programs

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We present the experience of the first 30 months of the Telethon Undiagnosed Diseases Program (TUDP), a pilot program devoted to unsolvable pediatric patients with complex syndromes. Pediatric patients with genetic complex disorders who went undiagnosed through array-CGH, biochemical and specific genetic tests, are discussed and prioritized in clinical plenary meetings. Patients may be recruited also by web form at http://www.telethon.it/cosa-facciamo/malattie-senza-diagnosi. Human Phenotype Ontology is used for phenotype description. Selected patients and parents (mostly as trios or quartet) are studied by high coverage (150x) WES. From June 2016 to December 2018, twelve national pediatric centers have presented 503 undiagnosed families. Fifty-two have been considered low-priority since outside inclusion criteria (i.e. severity, pediatric age, complexity). Of the 451 enrolled families, 196 have completed the entire diagnostic NGS workflow and final result has been provided. In 71 families (36%) we detected causative variants in known genes, mostly extending the phenotype appearance. Forty-six (65%) are de novo mutations, while 16 are autosomal recessive, 8 X-linked and one autosomal dominant with variable expression. In 67 trios (34%), we identified a strong candidate gene, while the pathogenic role of specific genetic variants is under investigation. In 58 trios (30%) no putative causative or candidate variant was identified. A fraction of families shares different mutations in the same genes, such as *DDXD3 and ASXL3* (in three families) and *EEF1A2, GRIN1, IRF2BPL, RARS2 and SMAD4* (in two families). Selected WES-negative cases are being studied by WGS or by linked read WES using 10x technology. Telethon Grant: GSP15001

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P11.81A

A homozygous *VPS26C* nonsense variant is associated with a novel syndromic phenotype

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Introduction: Novel fully penetrant monogenic disorders are presumably very rare. Initial non-availability of unrelated patients is therefore likely. To still enable the molecular classification of such cases, several types of information need to be considered.

Materials and Methods: Two reportedly related individuals with a distinct syndromic phenotype were investigated. Following negative chromosomal microarray and exome sequencing in the proband, patients were subjected to whole genome sequencing. The variants from the duo were analysed in a comparative manner, and used to estimate the degree of relatedness.

Results: The major clinical findings in both patients overlapped; they included neurodevelopmental deficit, skeletal abnormalities and distinctive facial features. Variant filtering demonstrated a single unique shared homozygous high impact variant, c.178G>T (p.Glu60*), in *VPS26C*. Variation databases do not list individuals with bi-allelic truncating variants in this gene, which codes for a protein involved in membrane protein recycling. Detailed genealogy analysis suggested close relationship for the patients' fathers, but very distant relationship for the mothers. An overall low degree of relatedness of the two patients, as confirmed by comparative analysis of the genomes, implies a low likelihood for the genotype-phenotype association to be a chance observation.

Conclusion: Based on the genealogical information, clinical findings, and comprehensive consideration of the genetic data, we propose *VPS26C* as a novel disease gene in humans. Consistent with widespread expression of *VPS26C* and with the fundamental biological function of the encoded protein, its bi-allelic inactivation causes a wide range of symptoms involving multiple organs.

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P11.82B

Diagnostic microdeletion/microduplication detection by exome sequencing enabling copy number variation analysis

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Introduction: We present two cases where copy number variation (CNV) analysis by exome sequencing revealed a microdeletion and a microduplication, respectively, explaining the under-investigation phenotypes.

Materials and Methods: A 31 y.o. pregnant woman with mild symptoms of Waardenburg Syndrome (WS) and a 35 y.o. woman with absence of the uterus were tested. Clinical exome sequencing was performed using Sophia Genetics' Clinical Exome Solution v2, which includes 4,493 genes. Following preparations according to the manufacturer's protocol, DNA libraries were sequenced on an Illumina NextSeq-500 genetic analyzer. Data processing, variant calling and CNV analysis were conducted by SOPHiA DDM® bioinformatics pipelines.

Results: The analyses revealed a *PAX3* gene deletion, confirming WS in the first patient and a duplication, including at least *SCARF2, SERPIND1, SNAP29* and *CRKL genes*, consistent with Mayer-Rokitanski-Kuster-Hauser syndrome, in the second patient. Both CNVs were ascertained and their exact size determined (≈725kb and ≈1.05Mb respectively) by arrCGH.

Discussion: Until recently, exome sequencing rose the diagnostic yield of genomic investigations by revealing SNV/INDEL disease-causing variants. The design and analysis pipelines of the exome sequencing we implement empowers CNV detection as well. We believe that in the near future, technology and bioinformatics advancements may permit CNV analysis as effectively as with well-

established approaches thus, facilitating more efficient genetic diagnosis and healthcare management.

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P11.83C
Exploring WAGR syndrome: genotype-phenotype associations in the 11p13 region

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Introduction: WAGR syndrome (Wilms' tumor, aniridia, genitourinary malformations and impaired mental development) is associated with deletions of chromosomal region 11p13. Recently, some evidence accumulated on microdeletions within 11p13 with varying clinical manifestations. We report two such cases, and tentatively outline possible genotype-phenotype associations in the region.

Patients and Methods: Our first case featured normal intellectual ability, pseudohermaphroditism, acute lymphoblastic leukemia, Wilms tumor, glomerulonephritis and cerebellar angioblastoma. The second patient had complex genitourinary malformations, global developmental delay and mental deficit. RT-PCR was used to detect gene copy numbers in the 11p13 region in both cases. FISH and a dPCR-based analysis were performed in case 2.

Results: In patient 1, a heterozygous deletion was found affecting the whole WT1 gene but neither one of the flanking genes. In patient 2, a microdeletion was seen in the 11p13 region affecting only WT1 and EIF3M. In this case, we also detected a deletion at 22q13.

Conclusions: While WAGR syndrome is generally considered as a single clinical entity caused by a relatively large deletion, the seemingly high frequency of microdeletions in the area (as seen in our cases and various anecdotal reports in the literature) suggests that it may represent a spectrum of clinical features, the observed phenotype depending on which genes happen to be deleted. WT1 seems to be responsible for genitourinary tumors and malformations, while in cases featuring a PAX6 or BDNF deletion, we may observe aniridia or obesity, respectively. Genes PRRG4 and SLC1A2 are likely candidates for causing intellectual deficit when deleted.

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P11.84D
Whole exome sequencing reveals a homozygous mutation in *NBAS* in a critically sick newborn with a complex immunologic phenotype

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We report a newborn with a multisystemic disease severely affecting immune and gastrointestinal systems and skin. He was admitted to Neonatal Intensive Care Unit because of growth failure, recurrent infections with sepsis and dehydration, hypogammaglobulinemia, absence of B lymphocytes, reduced NK cells, ichthyosis, hepatosplenomegaly and facial dysmorphism (proptosis, thin lips, loose skin and reduced subcutaneous fat). At a first glance, clinical presentation made us think about a syndromic immunodysregulation; we performed whole exome sequencing (WES) which identified a biallelic variant in *NBAS*. Neuroblastoma amplified sequence (NBAS) gene encodes a protein highly expressed in connective tissue, eye, brain and spinal cord, and it is involved in nonsense-mediated mRNA decay. Mutations in NBAS cause disregulation of genes involved in instruction of inflammatory response. Biallelic mutations in this gene have been associated with phenotypic spectrum that ranges from isolated acute liver failure in infancy to a multisystemic condition including short stature, optic nerve atrophy and Pelger-Huet (PH) anomaly of granulocytes (SOPH). Moreover, biallelic variants were reported recently in two unrelated patients with characteristic facial appearance, PH anomaly and severe hypogammaglobulinemia with frequent infections. Clinical exome sequencing uncovers monogenic disorders in a significant number of infants in NICU when suspected to have genetic disorders, significantly influencing the medical care. Performing exome sequencing as first-line test can achieved over three times diagnosis rate, with less than one-third of the cost, compared with traditional tiered testing strategy of single gene or gene panels.

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P11.85A
Family trio analysis in rare syndromes - Clinical experience and outcome

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Whole exome (WES) or whole genome (WGS) sequencing in trios is becoming a common molecular diagnostic tool for analysis of rare genetic disorders.

At Clinical Genetics, Karolinska University Hospital, clinical trio analysis has increased tenfold from 2012 - 2018. In total, trio analysis has been finalized for 503 patients with undiagnosed, suspected rare genetic conditions. Data was analyzed for sequence variants in the coding part or splice regions of the OMIM genes that were consistent with Mendelian inheritance. In general CGH-array and FRAXA analyses had been performed on the patients prior to trio analysis.

Of the 503 cases, the molecular diagnosis rate was 33%. *De novo* heterozygote sequence variants were the most prevalent causes of genetic disorder, 55%. Sequence variants consistent with autosomal recessive inheritance were detected in 32% and X-linked recessive inheritance in 8% of the cases. In eight cases, an autosomal dominant inherited sequence variant was identified - explained by a known dominant trait or variable expressivity. Most patients were referred regarding intellectual disability and/or a syndromic phenotype. The different detected genetic diagnoses are rare, and for most cases the genetic outcome could not have been predicted.

In conclusion, trio analysis in families with an unknown rare disorder has proven to be a valuable tool to obtain a genetic diagnosis. The identification of the genetic aberration in these patients is crucial for estimation of recurrence risk. It is also a pre-requisite for carrier testing, prenatal- or preimplantation diagnostics.

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P11.86B
Whole exome sequencing reveals novel mutations in a cohort of undiagnosed patients with multiple malformation syndromes

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Multiple malformation syndromes (MMS) represent a heterogeneous group of genetic disorders associated with developmental anomalies in two or more systems. Using whole exome sequencing (WES) our ongoing study aims to molecularly characterise a cohort of undiagnosed Cypriot MMS patients. Recruitment criteria included: normal karyotype and array-CGH analyses and negative results for fragile-X syndrome and targeted gene testing. The study also aims to develop an in-house population-specific allele frequency database.

Family-based WES was performed in 21 families (67 individuals) comprising 26 MMS patients, using Illumina's TruSeq DNA exome library preparation kit and the NextSeq500. Bioinformatics analysis was carried out using an in-house pipeline according to the Genome Analysis Toolkit Best Practices. Possible pathogenic mutations were confirmed by Sanger sequencing followed by segregation analysis.

NGS analysis revealed 11 potentially pathogenic variants, including 9 novel and 2 known variants, achieving an overall detection rate of 42% (11/26 patients). Flagged variants were found in *PCNT, SPR, PXDN*, *COL27A1*, *UBE3A*, *KAT6A, KDM6A, POMGNT1* and *PIEZO2*, causing microcephalic osteodysplastic primordial dwarfism type II, sepiapterin reductase deficiency, anterior segment dysgenesis of the eye, Steel syndrome, Angelman syndrome, intellectual disability, Kabuki syndrome-2, muscle-eye-brain disease and distal arthrogryposis, respectively.

In conclusion, this study has identified 9 novel mutations in known genes and has expanded the phenotypic spectrum associated with these genes. This study also reports the first *COL27A1* mutation in the Caucasian population. The detection rate of 42% indicates that WES is a reliable and cost-effective tool for identifying the molecular basis of MMS. Further functional studies are ongoing.

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P11.87C
The utility of reverse phenotyping using whole-exome sequencing in an undiagnosed infant with neurological symptoms

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Aim: Whole-exome sequencing (WES) is being used increasingly to diagnose rare complex diseases. The purpose of this study is to determine the contribution of reverse phenotyping in diagnosing diseases with blended phenotypes.

Method: A 22-month-old girl was referred to our clinic with developmental delay, hypotonia, convulsion and vomiting. She was the first child of consanguineous parents. On physical examination she had growth retardation, hypotonia and dysmorphic facial features. Her laboratory tests were normal. A brain MRI revealed a white matter hypomyelination.

Result: A specific diagnosis could not be established via clinical features and laboratory tests, however WES revealed homozygous mutations in three different genes. The variant c.3412_3418del (p.Val1138Metfs*25) in *CNTNAP1* gene has been classified as likely pathogenic, c.1090G>A (p.Val364Met) variant in *DPYS* gene classified as a variant of uncertain significance (VOUS), and c.2480G>A (p.Arg827Gln), in *ATP7B* gene classified as a VOUS in accordance with ACMG 2015 criteria. The parents were found to be heterozygous for the same mutations.

Disscussion: Following WES results, clinical compatibility was investigated. Developmental delay and hypomyelination in the patient were suggestive of Congenital Hypomiyelinating Neuropathy caused by *CNTNAP1* mutations. Persistent vomiting is a symptom of Dihydropriminidase deficiency caused by *DPYS* mutations. A decreased level of ceruloplasmin in the patient is considered as the first sign of Wilson's disease caused by *ATP7B* mutations.

Conclusion: Reverse phenotyping using WES shortens the diagnostic process, identifies the additional diseases in patients with blended phenotypes and improves the quality of genetic counseling.

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P11.88D
BAZ1B is a candidate gene responsible for hypothyroidism in Williams syndrome

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Williams syndrome (WS) is a rare neurodevelopmental disorder affecting 1/7500 live births resulting from a hemizygous deletion of 28 genes on chromosome 7q11.23. This condition is characterized by mild to moderate intellectual disability or learning problems, unique personality characteristics, distinctive facial features, and heart and cardiovascular problems. Moreover, WS subjects frequently suffer of several endocrine abnormalities including hypothyroidism, idiopathic hypercalcemia, early puberty, impaired glucose tolerance. Full hypothyroidism occurs in about 10% of WS subjects; however, subclinical hypothyroidism occurs in about 30% of patients. Several data indicate that the hypothyroidism is due to thyroid morphology defects: thyroid hypoplasia has been found in a large fraction of WS patients suffering of full or subclinical hypothyroidism. Several genes involved in thyroid dysgenesis have been identified; however, none of them is in the 7q11.23 region. Thus, the hypothyroidism molecular bases in WS are not known. By a microarray approach we analyzed the expression levels of 7q11.23 region genes in embryo mice and selected those expressed also in thyroid or involved in its development. Among them, BAZ1B, a member of bromodomain protein family, appeared particularly relevant. Silencing, by RNA interference, BAZ1B in a normal thyroid cell line (Nthy-ori-3.1) and in two anaplastic thyroid cancer derived cell line (SW1736 and 8505C), we observed a significant cell viability reduction (about 50% in both cell lines), demonstrating the importance of BAZ1B for thyroid cell proliferation. These results demonstrate the relevance of BAZ1B loss in thyroid cells proliferation, suggesting a correlation between its deletion and thyroid hypoplasia in WS.

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P11.89A
A new phenotype associated to an unreported *ZFPM2* variant: call for additional cases

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Introduction: *ZFPM2* is a gene coding for a zinc finger protein. It is a transcription factor involved in lung, diaphragm and cardiac development regulating the GATA family proteins. Three distinct phenotypic entities related to *ZFPM2* have been described: disorders of sex development, non-syndromic diaphragmatic hernia and congenital cardiopathies.

Material and Methods: We report on a patient presenting with major facial features associated with a polymalformative syndrome and a novel truncating *ZFPM2* variant.

Results: This 31-year-old patient presented at birth with diaphragmatic anterior eventration, pulmonary stenosis and right ventricular hypertrophy. He also had growth deficiency, micropenis, cryptorchidism and a shawl scrotum. He had a coarse face, hypertelorism, anteverted nares, a long philtrum, macrostomia, gingival hyperplasia and teeth agenesis. He was in a school for special needs, but intellectual deficiency was ruled out. He developed a dilatation of the ascending aorta in adulthood. Multiple investigations were normal. Exome sequencing revealed a frameshift insertion in *ZFPM2* (c.621delA; p.Ala208Leufs*5), not inherited from the father (mother not available).

Conclusion: The patient presents a peculiar association of malformations that belong to the ZFPM2 spectrum, but usually in a non-syndromic context. Such observations are needed in order to be able to describe a novel *ZFPM2* associated phenotype.

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P12
Cancer genetics

P12.001B
Mutational profiling of pediatric acute myeloid leukemia subtypes in the absence of recurrent chromosomal aberrations

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Introduction: Recurrent cytogenetic aberrations leading to the formation of fusion genes are found in more than 40% of pediatric acute myeloid leukemia (AML), while in approximately 20% of the patients their blast cells display cytogenetically normal karyotype (NK-AML). The aim of the work was to investigate mutational profile of AML patients without known chromosomal translocations regarding to immunophenotype data and clinical features.

Patients and Methods: The 34 patients from Uzbekistan with AML without known recurrent chromosomal aberrations were investigated (18 boys and 16 girls, mean age 10.6 years). The coding regions of 26 genes involved in the pathogenesis of AML were captured with SeqCap EZ Target Enrichment System (NimbleGen, Roche) and sequenced using Illumina's MiniSeq platform. The mutations were verified using Sanger sequencing.

Results: Most patients (20 from 34) had one or more non-synonymous mutations. One patient with M4 variant had biallelic CEBPA mutation, two patients had previously undescribed ETV6 mutations. Also, known somatic mutations were revealed in the following genes: NPM1 (8.8%), JAK2 (5.9%), IDH1 (5.9%), NRAS (8.8%). In addition, rare germline variants with minor allele frequency less than 1% were found in CUX1, FLT3, TET2, PTPN11 and NUP98 genes, that may indicate their role in genetic susceptibility to pediatric leukemia.

Conclusion: The data may contribute to understanding the mechanisms of leukemogenesis in pediatric AML in the absence of known fusion genes. The work was supported by the Russian Science Foundation (grant # 18−15-00398).

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P12.002C
Integrative analysis of miRNA and mRNA paired expression profiling of androgen-dependent and -independent prostate cancer cells treated with anticancer imidazoacridinone C-1311

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Introduction: Prostate cancer remains one of the most common cancer in the male population. Imidazoacridinone C-1311 (Symadex™) is a new inhibitor of topoisomerase 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 miRNA and mRNA integrated profiles of prostate cancer cells with different expression of androgen receptor (AR).

Materials and Methods: Prostate cancer cells were exposed to C-1311 for 24h. The miRNA and mRNA expression profiles (adj. $p < 0.05$ and log fold change > 1) were generated from total RNA using nCounter Nanostring platform and RNA-seq using Illumina HiSeq 4000 platform, respectively. MiRNA-mRNA expression pairing and pathways analysis was performed using Ingenuity Pathway Analysis software.

Results: Paired genomic analysis identified a total of 33 miRNA-mRNA pairs in LNCaP (AR+) and 65 miRNA-mRNA pairs in DU-145 (AR-) cells. Selected miRNAs and mRNAs associated with tumor formation and progression, cell cycle or apoptosis (including miR-542, miR-138, miR-590, miR-125, miR-34, BIRC5, CDKN1A, CDC25A, KNTC1) were validated by qPCR. Paired miRNA-mRNA profiles revealed activation of diverse signaling pathways depending on AR status. In LNCaP (AR+) cells major changes were found for genes involved in cell cycle control, p53 signaling and DNA damage response, whereas pathways enriched for DU-145 (AR-) cells were involved in cancer-related inflammation and cellular metabolism.

Conclusions: Our study identified C-1311 induced miRNA candidates which may be effective targets for future prostate cancer therapy. Founded by the National Science Centre, Grant No 2013/09/D/NZ7/04185.

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P12.003D
Study of androgen-receptor pathway in breast cancer cell lines: a new potential target

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Introduction: Breast and prostate cancers depend on steroid hormones (oestrogens, androgens) to stimulate their growth. This makes hormonal pathways prime targets to treat these kinds of cancer. Counter-intuitively, it has been shown that the androgen receptor (AR) is expressed in approximately 80% of breast cancer patients and over 30% of triple-negative breast cancer patients. Thus, we aim to study the effects of androgen receptor-inhibitor enzalutamide on breast cell lines

Material and methods: HCC-1937, HCC-1569, MCF-7, Hs578T, HBL100, BT-549, MDA-MB231, AU-565, MDA-MB-415, BT-474 (breast cancer cell lines) and VCAP (androgen receptor-positive prostate cancer cell line used as a positive control), were used to extract RNA and proteins. Expression of AR and variant 7 (AR-V7) was confirmed by RT-PCR, droplet digital PCR and simple western analyses. MTT viability assays with enzalutamide (50, 75 μM) were performed.

Results: The PCR and simple western results are shown in the table below. MTT assays show growth inhibition at enzalutamide concentrations of over 50uM in BT-474, MDA-MB-231, Hs578T, MCF-7 and MDA-MB-415.

Cell Lines	ER	PR	HER2	TP53	AR	AR-V7	Simple Western AR	MTT response
HCC-1937	−	−	−	−	−	−	−	NA
HCC-1569	−	−	+	−M	+	−	−	NA
MCF-7	+	+	−	+/−WT	+	−	+	+
Hs-578T	−	−	−	+M	+	+	+	+
HBL-100	−	−	−	++	+	+	−	NA
BT-549	−	−	−	++M	+	+	+	NA
MDA-MB-231	−	−	−	++M	+	−	+	+
AU-565	−	−	+	+WT	−	−	+	NA
MDA-MB-415	+	−	−	+	+	+	+	+
BT-474	+	+	+	+	+	+	+	+

Conclusions: Androgen inhibitors could be a potential therapeutic target for androgen receptor-positive breast cancers.

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P12.004A
Mosaic *APC* mutations in patients with mild polyposis phenotypes

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Introduction: Mosaic mutations in the *APC* gene have been identified as a common cause (25%) for polyposis in patients with >20 adenomas and no germline mutation. The frequency remains unknown in patients with milder phenotypes.

Materials and Methods: The *APC* gene was sequenced in DNA isolated from 4 adenomas in a cohort of polyposis patients (n=120) using Next Generation Sequencing. Patients were considered mosaic if an identical mutation was identified in all lesions. Detection rates were compared between subgroups according to the number of adenomas. Clinical characteristics were compared between those with and those without a mosaic mutation.

Results: The mosaicism detection rate was 18% in the whole cohort (22/120), 0% in patients with <10 adenomas (0/18), 9% in those with 10-20 adenomas (3/34) and 12% in patients over age 70 (2/17). Some patients were found to have an identical variant in only a subset of adenomas (n=21) and were called hybrid cases. Mean age of diagnosis was comparable in hybrid and non-mosaic cases (both 62 years) and lower in pure mosaic cases (50 years). Number of adenomas was also comparable between hybrid and non-mosaic cases (n=22 versus n=18) and higher in mosaic cases (n=38)

Conclusions: Our results indicate that mosaic *APC* mutations also play a role in patients with less than 20 adenomas and/or older age at presentation. Further expansion of the cohort is needed to validate our results and to elucidate the cause and clinical significance of hybrid mosaic mutation patterns. Dutch Cancer Society project 11292

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P12.005B
An ALK inhibitor, AZD3463 improves the anticancer effects of rapamycin in breast cancer cells

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Introduction: Breast cancer is the most common type of neoplasia in women and rapamycin analogues are frequently used in current treatment protocols. The limitations of the use of rapamycin in therapy include the activation of the PI3K signaling pathway by other signaling pathways and reactivation via feedback regulation. AZD3463 is an anticancer agent used as a potential ALK/IGF1R inhibitor and it has been determined that ALK inhibitors induce PI3K/AKT/mTOR-mediated apoptosis and autophagy. In this study we investigated the *in vitro* effect of the rapamycin-AZD3463 combination on breast cancer by providing lateral inhibition of PI3K signaling pathway.

Material Methods: Cytotoxic, apoptotic, autophagic and cytostatic effects of rapamycin, AZD3463 and combination on MCF7 cell line were assessed by the WST1, Annexin V and JC-1, Tb/GFP TR-FRET LC3B Expression, Cycletest assays in a dose-and time-dependent manner. Expression level alterations of PI3K/Akt/mTOR pathway related genes in treated MCF7 cells were evaluated by quantitative Real-time PCR (qRT-PCR), compared to control cells.

Result: The effects of AZD, rapamycin and combination on cell viability, apoptosis, autophagy, cell cycle and gene expression levels in MCF7 cell line are summarized in the table.

	AZD3463	Rapamycin	Combination	Comment
IC_{50} Values	765nM (72^{nd})	10.03nM (72^{nd})	AZD-0.3nM Rapa-28.85nM (72^{nd})	A synergistic effect was observed in the combination.
Annexin V Assay (Fold change relative to a control)	(+) 3.6	(+) 1.2	(+) 7.4	It was determined that apoptosis increased in combination according to separate applications.
JC-1 Assay (% change of mitochondrial membrane potential)	(+) 0.04	(+) 5.5	(+) 48.2	Combination application increased mitochondrial membrane potential.
Cell Cycle Assay (Fold change of check point arrest)	G_0/G_1 (+) 2.4 S-1.05 G_2/M- (-) 2.7	G_0/G_1- (+) 2.8 S-1.12 G_2/M- (-) 4.7	G_0/G_1- (+)2 S-1.2 G_2/M- (-) 1.8	Rapamycin, AZD3463, and combination have been found to effectively induce G0/G1 arrest.

Autophagy Assay (Fold change of autophagosome accumulation)	(+) 3.84	(+) 3.31	(+) 4.17	Combination application in terms of autophagic effects is more effective than individual doses.
Gene Expression Analysis (PI3K/AKT/mTOR pathway related genes)	WASL (-) 5,7 RHOA (+)5,4 FOS (+) 0,19	WASL (-) 4,5 RHOA (-) 0,9 FOS (+) 2,0	WASL (-) 12,5 RHOA (-)4,2 FOS (-) 4.2	It was determined that the combination application was more effective than the other groups.

Conclusion: In this study, we suggest that the combination of rapamycin-AZD3463 can increase the efficacy of treatment by overcoming factors limiting the use of rapamycin in the treatment by simultaneous inhibition of ALK / IGF1R pathway.

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P12.006C
BAP1 loss of protein expression: an analysis for *BAP1* germline Variants of Unknown Signification (VUS) interpretation

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The *BRCA1-associated protein-1* (*BAP1*) tumor-predisposition syndrome (*BAP1*-TPDS) is a hereditary cancer syndrome with increased risk to develop a variety of cancers including four core tumors types, uveal and cutaneous melanomas, mesotheliomas and renal clear cell carcinomas (Walpole *et al*. 2017). Worldwide, 40 different missense variants were identified, but only 9 were classified as likely pathogenic upon modified ACMG criteria (higher weight for phenotypical evidence for rare tumors). *BAP1* gene being a tumor suppressor, genetic inactivation leads to loss of nuclear expression by immunohistochemistry (IHC). The frequency of inactivation by two somatic genetic events varies upon tumor types; therefore, the specificity of BAP1 loss of staining to indicate a germline *BAP1* mutation (positive predictive value, PPV) varies also.

In the present study, we performed 217 BAP1 IHC (145 of them in cutaneous melanocytic tumors) in 118 members of 102 families, analyzed in parallel for *BAP1* germline mutations to address the interest of BAP1 IHC to the *BAP1*-TPDS diagnostic. Given the variable PPV upon tumor types, only results of the 28 families with at least BAP1 IHC for two independent tumors are presented in the table below:

Number of tumors with BAP1 loss	Number of tested tumors	Number of families	*BAP1* genetic test results
1	2 to 7	7	6 wild type 1 class 3 variant (VUS)
2	2	9	8 class 5 variants (deleterious) 1 wild type
More than 2	3 to 21	12	9 class 5 variants (deleterious) 3 class 3 variants (VUS)

In conclusion, we propose that the presence of two lesions with BAP1 expression loss in a family (same or different patients) be considered as a Pathogenic Moderate criterion to classify *BAP1* variants under ACMG criteria.

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P12.007D
Insights in genetic mechanisms of aggressiveness of Basal Cell Carcinoma

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Vismodegib is a smo inhibitor approved for the treatment of advanced basal cell carcinoma (BCC) with activated Hedgehog pathway. Most patients show clinical benefit from the drug but a small subset shows intrinsic resistance (IR) and progresses on treatment. We report for the first time molecular mechanisms of IR in 4 aggressive BCC cases. Using whole exome sequencing, analysis of copy number aberrations and transcriptional profiling we were able to uncover the major determinants of IR in the studied samples. Our results show that these BCCs harbor either molecular signature earlier reported in BCCs with acquired resistance to vismodegib, such as p.W535L SMO mutation and amplification of GLI2; or bear molecular signature compatible with squamous cell carcinoma evolution (mutations and focal copy number alterations of MYC, LGR4 and TCF7L1). In line with the detected driver events at DNA level upregulation of target genes of either Hh or WNT

pathway was confirmed with RNA sequencing. Additionally we report that all the BCC IR cases overexpress TRAG3, SNORA42 and microRNA711, markers of poor prognosis and resistance to treatment which were previously identified in other types of cancer.

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P12.008A

Molecular signature for improvement of clinical prognosis accuracy in superficial bladder carcinoma

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Background: The aim of our study is to identify the correlation between molecular changes in uroepithelial tumors and tumor stage, grade and progression, as well as to evaluate potential prognostic markers.

Materials and Methods: Tumour samples from 65 Bulgarian patients, staged pTa, pT1, pT2, pT2a, pT2b, pT3 and pT4 were collected. Four genes (*AP1S1, FIGF, HDAC11, CDK9*) that showed significant difference between invasive and non-invasive bladder carcinoma in a previous pool analyses were selected for individual validation by RT-PCR analysis (Qiagen). CNVs of 30 uroepithelial neoplastic samples (CytoChip Oligo aCGH, 4x44K and Infinium OncoArray-500K BeadChip, Illumina) were performed. Data was analyzed by BluefuseMulti software and Karyostudio.

Results and discussion: The gene expression analysis revealed 4-12 fold change difference in the expression level between pT2 and pTa and pT1 tumors. The highest up-regulation was shown for *AP1S1* gene, involved in protein trafficking by clathrin-coated vesicles. Five LOH variants in two pT2 samples were determined: arr 6p12.1p11.1 (55721511-58767335)x2hmz, arr (7q21.3(94372640-97676259), 7q22.1q22.2(99552168-104336782))x2hmz, arr (11q14.1q14.2(81417643-86071005), 17q25.1q25.3 (73593574-77382564))x2hmz. No chromosomal abberations were found only in two pTa samples. Genomic imbalances – losses were mostly found for arr 6p21.32 (32453988-32538289)x0 and arr 9p21.3(20351075-22755025)x0. CNVs gains were identified in almost all chromosomes, but the most frequent were in chromosome (9)(q21.11q21.13), (9)(q31.2q31.3), (20)(q11.21q13.33) and (20)(p13p11.1) mainly in pT1 and pT2 tumours. Unexpectedly in pT3 and pT4 tumour samples only gains in chromosomes:1p, 11q, 17p and 17q were detected.

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P12.009B

Cytogenetic abnormalities in B-precursor acute lymphoblastic leukemia from a tertiary care centre in India

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Introduction: B-precursor acute lymphoblastic leukemia (BCP-ALL) is a neoplastic disease characterized by clonal expansion of leukemic cells and cytogenetics is an important diagnostic parameter in its classification.

Material and Methods: Retrospective study was carried out in 2270 patients (Adult- 759; Pediatric- 1511) diagnosed as BCP-ALL (January 2016 to December 2018). Fluorescence *In Situ* Hybridization (FISH) was performed using commercial probes for LSI *BCR/ABL1; MLL; ETV6/RUNX1; TCF3/PBX1* and centromeric probes for chromosomes 4, 10 and 17 for ploidy status. A pilot study was conducted for Ph-like ALL (FISH was performed for *ABL1,*

ABL2, *PDGFRβ*, *CSF1R* and *CRLF2* rearrangements) on 219 cases (June 2018 to December 2018).

Results: Cytogenetic abnormality was detected in 51% cases (1162/2270) [Adult: 60% (454/759); Pediatric: 47% (708/1511)]. Incidence of t(9;22); *BCR-ABL1*, t(1;19); *TCF3-PBX1 and MLL* translocations was 31%, 4% and 3% in adult B-ALL while 6%, 7% and 3% in pediatric B-ALL respectively. Translocation (12; 21): *ETV6/RUNX1* was found in 13% pediatric cases. Trisomy 4, 10, 17 were detected either as a sole or in combination with each other in 31% (462/1511) pediatric cases and 22% (165/759) adult cases. Chromosomal counting revealed hyperdiploidy and hypodiploidy in 25% and 5% pediatric cases; 12% and 6% in adult cases which was correlated with flow cytometry DNA index. Of 219, 4 cases (1.8%) showed rearrangement in *CRLF2* (1 Pediatric case with Down syndrome) and *CSF1R* (Adult-1; Pediatric-2). Follow-up studies were performed.

Conclusions: Various cytogenetic subtypes have significant impact on risk stratification and hence remain strong independent indicators of disease outcome.

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P12.010C
Constitutional mosaicism of BRCA2 gene mutation detected by massive parallel sequencing in a case of early onset breast cancer

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Pathogenic variants in BRCA1 and BRCA2 genes are responsible for over 50% of genetic susceptibility to breast and ovarian cancer. Nowadays, the screening of those genes is commonly performed with expanded panel genes using massive parallel sequencing (MPS). This technology is more sensitive and improves the capacity for detection of mosaic events. Very few cases of *de novo* mutations have been described in BRCA genes with only one constitutional mosaicism of BRCA1 mutation demonstrated by Friedman and coll. Here, we report the first case of constitutional mosaicism of BRCA2 mutation in a 46 years old woman referred to genetic counselling for bifocal lobular breast cancer. Two sisters developed breast cancer at 74 and 58 years old. Any cancer wasn't diagnosed in upper generation. We perform genetic testing of a large panel of breast cancer susceptibility genes by MPS on a blood sample. The c.4688G>A, p.(Trp1563*) BRCA2 mutation was detected in 23% of sequence reads (>500X depth coverage) suggesting mosaic condition. Mosaic rate was confirmed in other tissues as bladder cells and salivary sample. Higher rate was found in capillary bulb. The analysis of the non-cancerous breast tissue is in progress.

Finally, this case suggests that, although rare, this event should be taken into account in the evaluation of high-risk families and it is probably underestimate. Two reasons should increase the number of cases reported: large screening performed by highly accurate MPS and enhanced BRCA testing (outside of the fulfilled high-risk selection criteria) because of targeted therapies.

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P12.011D
The *BRCA1* intronic c.5407-25T>A variant: effect on splicing and association with breast and ovarian cancer

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Introduction: The increasing genetic testing for hereditary breast and ovarian cancer leads to detection of rare sequence variants of unknown clinical significance in the *BRCA1* and *BRCA2* genes. Correct interpretation of these variants is challenging, but important for correct clinical management. The aim of this study was to determine the pathogenicity of the intronic *BRCA1* c.5407-25T>A variant found in Norwegian and French families.

Results: The *BRCA1* c.5407-25T>A variant was detected in 17 families with suspected hereditary breast and ovarian cancer (mean Manchester score 16.1). This is a rare variant with a reported frequency of 0.00001579 in non-Finnish European population in the gnomAD database. It was found in 1/370 anonymous Norwegian blood donors and 0/784 patients included in an in-house diagnostic exome database. NGS-based RNA sequencing of *BRCA1* in blood revealed that the variant leads to skipping of exon 22 and frameshift

predicting the truncated BRCA1 protein p.(Gly1803GlnfsTer11). This exon skipping was confirmed by PCR and Sanger sequencing of the *BRCA1* mRNA booth in blood, breast and in ovarian tissue. However, sequencing of two c.5407-25T>A carriers heterozygous for a transcribed SNP (c.4837A>G), showed that a low amount of correctly spliced transcript including exon 22 is generated from the c.5407-25T>A allele. Western blot analysis of transiently expressed BRCA1 proteins in HeLa cells showed a reduced amount of the mutant p.Gly1803GlnfsTer11 compared to wild-type protein.

Conclusion: Our results indicate that *BRCA1* c.5407-25T>A is a likely pathogenic splice variant associated with hereditary breast and ovarian cancer.

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P12.012A

Functional studies of SNPs in glycosylase genes as modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers

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Introduction: In a previous association study, we found three SNPs in different DNA glycosylase genes (*OGG1*, *NEIL2*, and *UNG*) that modify breast or ovarian cancer risk in *BRCA1* and *BRCA2* mutation carriers.

Materials and Methods: We have used a series of 300 familial breast and ovarian cancer patients to try to gain molecular insights into how these variants exert their cancer risk modifier effect. In this series, we have measured glycosylase expression and activity and different genome instability and oxidative stress hallmarks to explore the role of these SNPs.

Results: The studied SNPs are associated with expression changes in their respective glycosylases. We have also found associations of the SNPs with DNA damage levels, telomere length or oxidative stress levels, specifically for the group of mutations carriers where the different SNPs respectively exert their cancer risk modifier effect.

Conclusions: Our findings help to explain the association of these SNPs with cancer risk in *BRCA1/2* mutation carriers, highlighting the importance of genetic changes in glycosylase genes as modifiers of cancer susceptibility for *BRCA1/2* mutation carriers. Grants: J.M.B. is supported by grant FPU15/01978 from the Spanish Ministry of Education, Culture and Sport.

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P12.013B

Impact of germline *BRCA* mutation identification on subsequent breast cancer stage, therapy and survival - implications for routine screening

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Background: Screening healthy Ashkenazi Jews for germline *BRCA1/BRCA2* mutations (g*BRCA*) is not standard policy, despite high (2.5%) carrier rates. Most carriers are identified only after breast cancer diagnosis.

Aim: to determine if pre-symptomatic knowledge of carrier status affects breast cancer stage and management.

Methods: Record review of g*BRCA* carriers (excluding women with risk-reduction mastectomy), diagnosed with breast cancer between 1/2005-4/2016. We compared outcomes between carriers whose g*BRCA* was identified pre-breast cancer (PRE-C-D) vs. post-breast cancer (POST-C-D) diagnosis.

Results: 105 women with breast cancer had g*BRCA:* 42 (40%) PRE-C-D and 63 (60%) POST-C-D. Mean age at diagnosis (50.4y) and *BRCA1:BRCA2* distribution (64%:36%) were similar in both groups. PRE-C-D carriers were significantly more likely to have suggestive family history (93% vs. 63%), prior breast cancer screening (78% vs 64%) and diagnosis by imaging (78% vs 25%) rather than clinical symptoms (19% vs 73%) ($p<0.001$, all comparisons). PRE-C-D carriers had lower stage at diagnosis ($p<0.001$), with no differences in tumor grade, hormonal receptor or Her2 status. PRE-C-D carriers were less likely to receive chemotherapy (34% vs. 94%), and more likely to

elect bilateral mastectomies (66% vs 17%) (p≤.001 all comparisons). In multivariate analysis, PRE-C-D g*BRCA* identification predicted for early stage (0-I) breast cancer diagnosis (OR=18.4, p<0.001). Initial analysis indicates better overall survival (HR=0.16, p=0.087)

Conclusions: Presymptomatic identification of g*BRCA* status is significantly associated with earlier stage breast cancer diagnosis, less extensive treatment, and possibly improvement in overall survival. This supports routine g*BRCA* testing in the Ashkenazi Jewish population. Funded by the BCRF

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P12.014C
Estimation of mutation frequency in new breast cancer susceptibility genes in a Greek patients' cohort

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Introduction: Many other than *BRCA1* and *BRCA2* genes have been implicated in breast cancer predisposition during the last decade enabling the identification of more women at higher risk for breast cancer compared to the general population and develop surveillance protocols tailored to their needs in the near future.

Purpose: The purpose of the current study was to estimate the prevalence of pathogenic mutations in the "new" breast cancer susceptibility genes in a Greek patients' cohort.

Patients and Methods: 104 women who developed breast cancer under the age of 55 years old with or without family history and women free of cancer but with two or more affected first degree relatives constitute our patients' cohort. DNA was extracted with Nucleospin tissue kit and libraries were prepared using the TruSight® Cancer Sequencing Panel, which includes 40 breast cancer susceptibility genes, following the manufacturer's instructions. Massive parallel sequencing was conducted on Miseq platform (Illumina).

Results: Two cases carrying missense mutations of unknown clinical significance were identified in *MLH1* and *PALB2* genes, respectively. One case harbors the *CHEK2* c.1100delC mutation, which confers three- to five-fold increased risk compared to the general population, while two women were heterozygote for pathogenic mutation in the *RAD51C* and *RAD51D* genes, respectively.

Conclusion: The overall occurrence of possibly pathogenic mutations in breast cancer susceptibility genes, excluding the ones detected in the highly penetrant *BRCA1* and *BRCA2* genes, is 4.8%. Thus, these genes seem to enable the identification of more clinical cases which may be benefited by closer monitoring.

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P12.015D
Detection of variants of pathogenic and uncertain significance in an Irish population using multi-gene panels for breast cancer risk

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Breast cancer (BC) is the second most common cancer worldwide. Twin studies estimate that >30% BCs are the result of hereditary factors. While hugely informative, pathogenic variants in *BRCA1* and *BRCA2* explain only 16-22% of hereditary BCs. Several high- and moderate-risk genes, and >100 single nucleotide polymorphism (SNPs) have been identified which explain 33% of hereditary BCs. Approximately 50% of the hereditary risk of BC is unknown. Next-generation sequencing (NGS) is a cost-effective method of investigating numerous genes in one assay. There are an abundance of clinical multi-gene panel tests aimed at assessing the inherited risk of BC ± ovarian cancer. This study aimed to investigate germline variants genes included on breast ± ovarian cancer risk panels in an Irish population. DNA from 90 BCs and 77 controls were sequenced for variants in 83 genes from clinical breast ± ovarian cancer risk panels. Variant calling was performed following GATK best practices, and prioritised as loss-of-function(LOF) and missense variants; further grading was performed using five *in silico* prediction tools. Excluding common/benign variants, 56 variants were identified in 37 genes. 36.5% and 39.2% of cases and controls carried ≥one variant. Nine variants were classified as pathogenic/likely

pathogenic; three occurred in *ATM, BRCA1*, and *CHEK2*. Eight novel variants were identified, including a frameshift variant in *NF1*. Six variants were identified in the Lynch syndrome genes *MLH1, MSH2, MSH6*, and *PMS1*. These results highlight the need for caution when considering the use of multi-gene testing in the clinic given the challenges of accurately predicting variant pathogenicity.

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P12.017B
Genetic models of breast cancer based on eight common genetic variants

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Introduction: This study analyses eight SNPs of low penetrant genes to detect possible importance in breast cancer (BC) risk.

Materials and Methods: The study consists of 317 women: 171 with breast cancer (57.06±11.60 years) and 146 without previous history of any malignancy (50.24 ±10.69 years). Major breast cancer histological type was DIC (76.0%), followed by LIC (7.8%). We genotyped eight genetic variants (rs4415084 *FGF10*, rs2981582 *FGFR2*, rs889312 *MAP3K1*, rs3817198 *LSP1*, rs3803662 *TOX3/ TNRC9*, rs2293554 *CASP8*, rs13387042 and rs13281615 *CASC21)* by High Resolution Melting method and validated by Sanger sequencing.

Results: General, dominant and multiplicative genetic models confirmed significant association with BC in the case of *FGFR2* (*C/T*) and *MAP3K1* (*A/C´*) polymorphisms (in all cases p≤0.03). Odd ratios for general model: CT=1.771, TT=1.953, AC´=1.760, C´C´=2.894. Odd ratios for dominant model: CT+TT=1.822 and AC´+ C´C´=1.868 and odd ratios of multiplicative genetic models *T*=1.517 (95%CI=1.083-2.131), *C´*=1.676 (p≤0.04). Logistic regression of interaction revealed the lowest OR for the combination of original homozygote genotypes (AA +CC+TT) of *MAP3K1*, *FGFR2* and *LSP1* genotypes (OR=0.024, 95%CI 0.001-0.306, p=0.011), indicating lower risk for those without risk alleles.

The risk allele genotype (CC) of *LSP1* (*T/C*) gene polymorphism showed solely ER-positivity (100%), heterozygotes with one risk allele (TC) were also mostly ER+ (90.6%). The risk genotype (GG) of *CASP8* polymorphism showed 100% of HER2+, 100% of PR- and the same 50% of ER+ and ER- (p=0.006, p=0.018, p=0.04).

Conclusion: Out of eight genetic variants analysed in our study, four were identified to associate with BC, namely within genes *FGFR2*, *MAP3K1, LSP1* and *CASP8*.

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P12.018C
Analysis of a CHEK2 variant in a family with an ATM mutation and multiple breast cancer cases

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We present analysis of a CHEK2 variant found in a family of Moroccan Jewish origin with multiple breast cancer cases. Of 11 sisters, 5 were diagnosed with breast cancer in their 60's; one had both papillary thyroid cancer (age 67) and pancreatic cancer (age 76). Their mother had breast cancer (age 70). Panel testing in four sisters revealed two germline variants: a known pathogenic mutation in ATM-p. Q1970X; and a variant in CHEK2 classified as being of uncertain significance - c.592+3A>T. (rs587782849, gnomAD allele frequency= 0.000032). This variant has been reported once to alter CHEK2 splicing (Kraus C et al, Int J Cancer, 2017). 8 sisters were tested. Of the 5 with breast cancer, 3 (ages 60-66) carried of both variants and two (ages 61, 67) carried only the CHEK2 variant. The sister affected with thyroid and pancreatic cancer carried only the ATM mutation, as did 2 unaffected sisters (ages 53, 67). CHEK2 c.592+3A>T was found in 2/234 (0.8%) Sephardi breast cancer patients (also of Moroccan origin), and was also observed in an unaffected woman of Balkan origin (age 36). cDNA analysis revealed two alternate transcripts, one lacking exon 4, and one lacking exons 4-5 (both out-of-frame, confirming the previous report). Semi-quantitative PCR indicated 50% reduction of the wild-type transcript. In summary, CHEK2 c.592+3 A>T is a rare splicing variant, which should be reclassified as mild pathogenic. In this family, the presence of two pathogenic variants in moderate-risk breast cancer genes may explain the high load of late-onset breast cancer.

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P12.019D
Deregulation of TRAIL apoptotic pathway mRNA expression in breast tumours

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Introduction: TRAIL apoptotic pathway constitutes a promising therapeutic target for cancer patients, due to high selectivity and low toxicity of TRAIL agents when administered in monotherapy/combination therapies. The aim of our study was to search for possible predictive biomarkers for patient stratification to ensure maximum treatment efficacy of TRAIL targeted therapy.

Materials and Methods: 88 breast cancer tissues were examined for relative mRNA expression of TRAIL and its receptors (*DR4, DR5, DcR1, DcR2*), using RT-PCR/ΔΔCt method. For statistical analysis SPSSv22 package was used. Death receptor (DR4, DR5) protein levels were analyzed by Western blot in a subset of cases.

Results: Elevated mRNA levels were observed in 11%, 19.3%, 23.9%, 11.6% and 12% of the cases for *TRAIL, DR4, DR5, DcR1* and *DcR2* genes respectively. Reduced mRNA levels were detected in 64.6%, 38.6%, 31.8%, 45.3% and 42.2% of the cases for *TRAIL, DR4, DR5, DcR1*, and *DcR2* genes respectively. TRAIL expression was correlated with molecular subtype (p=0,011), DR4 with N category (p=0,029) and tumor grade (p=0,015), DcR1 with tumor grade (p=0,001) and ER/PR expression (p=0,051). All receptors correlated with the prognostic stage (DR4 p=0,005, DR5 p=0,029, DcR1 and DcR2 p=0,006). The receptors' mRNA levels presented linear correlations and the strongest was found between DR4/DR5 (R=0,634, p<0,001).

Conclusions: A significant deregulation of TRAIL pathway components mRNA expression in breast cancer was observed in the present study. Multiple simultaneous expression patterns of TRAIL receptors emerged, underscoring the importance of patient selection using predictive biomarkers in order to achieve efficient TRAIL targeted therapy.

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P12.020A
Identification of significant network markers for breast cancer in Middle Eastern women using integrated transcriptomic and network analysis

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Introduction: Breast cancer is a major health problem in the world and it is the most common cause of cancer death among women. The integration of gene expression profiles and protein-protein interaction (PPI) network provides a better understanding of the molecular architecture of diseases. Recently, many researchers have invested efforts for identifying subnetwork biomarkers for cancer. It has been shown that these subnetwork biomarkers are more robust and reliable than single biomarker genes that are selected based on the gene expression data only.

Materials and Methods: We performed an integrated network analysis of gene expression data with the PPI to identify significant subnetwork biomarkers. In addition, we used the k-nearest neighbor (KNN) algorithm to define the classifier. We validated the classification performance on an independent gene expression dataset of breast cancer samples. Moreover, we performed survival analyses using different transcriptomic datasets that consisted of over 3,000 samples.

Results: We have identified four subnetwork biomarkers that significantly distinguished breast cancer patients from healthy controls. The identified classifier achieved 97% predictive accuracy between tumor and normal samples. Moreover, the survival analysis demonstrated that high expression of our biomarkers is associated with poor disease outcome.

Conclusions: Our results suggest that the network analysis coupled with genomic data may provide a robust methodology to identify key biological programs associated with breast cancer progression and invasion and may lead to

improved diagnosis, prognosis and therapeutic options. Funding: This study is funded by KFSHRC Research Grant (2110006 to DC).

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P12.021B
Integration of tumour sequencing and case-control analyses is a powerful tool for identification of cancer predisposition genes

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Background: The genetic causes of a large proportion of breast cancer families is unknown and while our targeted sequencing of over 12,000 index cases and controls has identified numerous strong candidates, individually mutations in these genes are rare. Our work highlights the fact that in isolation, case-control studies will remain substantially underpowered to establish a clear role for novel breast cancer predisposition genes and orthogonal validation approaches will be required. Genomic analysis of tumours from carriers of germline variants in candidate genes can provide powerful additional evidence for involvement of a gene in cancer predisposition through identification of "second hit" somatic inactivation events and characteristic "mutational signatures".

Results: We applied this approach *to assess the role of RAD51C* in breast cancer predisposition. Full exon sequencing of *RAD51C* in a case/control analysis identified a significant excess of loss of function variants in cases compared with controls (0.36% versus 0.04%; OR=8.67, 95% CI =1.89 to 80.52, P=9.87x10^{-04}). In addition, the association was highly significant among cases with ER negative (P=1.57x10^{-05}) or triple-negative cancer (P=7.33x10^{-06}), but not in ER positive cases. Tumour sequencing from carriers confirmed bi-allelic inactivation in all the triple-negative cases and this was associated with high levels of large-scale genomic alterations and a mutational signature 3, both indicative of homologous recombination repair deficiency.

Conclusions: This study provides compelling evidence that germline loss of function variants in *RAD51C* are associated with triple-negative breast cancer and demonstrates the substantial power of tumour sequencing to advance the discover new cancer genes.

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P12.022C
Genomic mapping of Crete via *BRCA1* and *BRCA2* mutation analysis

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Introduction: Germline *BRCA1/2* loss-of-function variants are linked to increased breast and ovarian cancer risk. Up to date, more than 5000 distinct *BRCA1/2* pathogenic variants have been reported, while their frequency and distribution varies between populations and ethnicities. In the Greek population, founder effects have been described, mainly involving *BRCA1* loss-of-function variants. However, certain geographical regions were underrepresented in previous studies. We therefore aimed to define and molecularly characterize the *BRCA1/2* mutational spectrum in Crete, the largest Greek island with unique demographic and cultural characteristics.

Materials and Methods: Patients of Cretan descent, with breast or/and ovarian cancer fulfilling NCCN guidelines were selected for genetic testing by NGS or Sanger sequencing, followed by MLPA. Haplotype analysis was subsequently performed to investigate potential founder effects of recurrent alleles.

Results: In total 291 patients (273 females, 18 males) were included. Forty-eight patients (16.5%) carried 21 different pathogenic variants in *BRCA1* (45.8%; 22/48) and *BRCA2* (54.2%; 26/48). The deleterious variants c.6842-2675_7008-5558del and c.7806-2A>T in *BRCA2* and c.5492del in *BRCA1* were recurrent and constituted 50% of all identified pathogenic variants. Haplotype analysis confirmed a founder effect for all three variants with a particular origin for each of them. Subsequent analyses indicate that these are Cretan founders, since they have not been detected in patients originating from other regions of Greece.

Conclusions: The *BRCA1/2* mutational spectrum of the Cretan population shows particular geographical distribution, influenced by strong founder effects. These findings

may facilitate a population-based genetic screening for identification of unaffected mutation carriers and primary cancer prevention.

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P12.023D
Putative non-coding regulatory driver discovery using unmatched tumor-normal samples

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We had previously developed a methodology using allele specific expression (ASE) as a proxy to discovering Genes with Allelic Dysregulation (GADs) impacted by non-coding somatic mutations, which requires matched healthy-tumour samples. Here, we describe a methodology to discover GADs using unmatched samples to compare tumor and reference RNAseq ASE (e,g. GTEx). Somatic mutations under selection for tumorigenesis and falling in non-coding regulatory regions of a gene tend to affect transcription in an allelic fashion that is observed as altered allelic imbalance between normal and tumor samples. Moreover, we also expect this gene to have altered gene expression for selection to occur. Hence, to detect non-coding regulatory drivers in tumorigenesis, discriminating genes under selection or under an eQTL effect, we devised three criteria to discover true driver genes. To test our method, we used colorectal cancer data from the SYSCOL project treating tumor and healthy samples as unmatched. We discovered702 genes passing all filtering criteria. Of them, 197 overlapped with the 373 GADs previously discovered with matched samples. Next, we used SYSCOL tumor and GTEx samples as control from colon sigmoid and colon transverse, discovering 612 and 689 GADs, respectively. Of these, 480 genes overlapped among all three unmatched analyses passing all criteria with 152 overlapping with the previously discovered GADs indicating that our methodology can detect regulatory drivers. Many of these have already been implicated in various cancers. Current work is validating our approach using prostate adenocarcinoma samples and other cancers and exploring the understudied non-coding regulatory drivers.

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P12.024A
Exome sequencing identified potential causative candidate genes for serrated polyposis syndrome

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Serrated polyposis syndrome (SPS) is a poorly defined colorectal cancer predisposition syndrome characterized by 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.

To uncover causative variants, the exomes of 49 SPS patients have been sequenced (Illumina HiSeq) using leukocyte DNA. For data analysis, the GATK- and the Varbank2-software were used. The germline variants were filtered for rare (biallelic: MAF≤1%, monoallelic: ≤0.1% according to gnomAD and an in-house-database) loss of function (LoF) variants. The pLi-score and reactome pathway analysis were used for further prioritization.

Biallelic LoF variants were found in 3 genes none of which being recurrently mutated. All in all, 548 genes harbored heterozygous LoF variants. 25 genes were recurrently mutated. 31/551 genes were extremely LoF intolerant indicated by pLi-score ≥ 0.9. 10/551 genes are involved in DNA repair, 12 in cell cycle checkpoint control, and one gene in programmed cell death. Most interestingly, different variants in two patients were found in one gene functioning in the canonical wnt signalling pathway, as does RNF43, in which pathogenic variants have been shown to be causal for SPS.

Exome sequencing identifies potentially causative germline variants underlying the susceptibility to SPS. The current work-up consists of screening of additional SPS patients for the most interesting genes, and the inclusion of missense variants as well as CNVs. Additionally, a rare variant analysis and burden tests will be conducted.

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P12.025B
The prognostic landscape of alternative transcript isoforms across human cancers

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Introduction: Through alternative start site usage and alternative splicing, individual genes commonly produce multiple mRNA isoforms that can code for proteins with similar, distinct, or even opposing functions. Most cancer expression studies evaluate expression at a gene-level, not accounting for the impact of multiple transcript isoforms arising from the same genetic locus.

Materials and Methods: Herein, we used clinical and RNA-sequencing data from 5642 tumours from 14 different cancers from The Cancer Genome Atlas to investigate associations between gene expression, at both the gene and isoform levels, and patient outcomes using Cox regression models. In addition, we used SNP and methylation data to analyze how coordinated genetic and epigenetic events may regulate these alternative isoform decisions through application of the sQTLseekeR algorithm and a methylation correlation analysis.

Results: We have found that many genes have alternative isoforms that associate with survival in opposite directions, unveiling a new molecular level with strong associations with patient outcomes. Isoform-level data, when compared to gene-level data, revealed an additional 6605 genes with isoforms significantly associated with survival in the 14 different cancers studied (|meta Z score| $Q<0.001$). Additionally, we have identified potential genetic (SNP) and epigenetic (methylation) mechanisms for these alternative isoform decisions.

Conclusions: We have shown that approaching gene expression as an isoform-level phenomenon provides a more sensitive approach to identifying signatures associated with patient outcomes. Moreover, the genetic and epigenetic regulation of these events reveals specific molecular biology that may contribute to individual differences in disease outcome, and should be further explored.

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P12.026C
Identifying sequence variants contributing to hereditary breast/ovarian cancer in patients with no identified *BRCA1* or *BRCA2* variants

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Introduction: Patients with hereditary breast and ovarian cancer (HBOC) are diagnostically tested for pathogenic variants (PVs) in *BRCA1* and *BRCA2*. However, in most HBOC families no PVs are detected. Currently, several additional genes were shown to be involved in increasing the cancer risk in HBOC patients. Accordingly, we investigated a Norwegian HBOC cohort in order to identify genetic risk factors for cancer in these patients.

Material and Methods: Hundred and one HBOC patients negative for pathogenic variants in *BRCA1/BRCA2* were screened for PVs in 94 genes using next-generation sequencing. We used Illumina TruSight rapid capture technology to prepare the Illumina TruSight Cancer targeted sequencing libraries, which were sequenced using an Illumina MiSeq System. The sequencing data were analyzed using Illumina MiSeq Reporter, and detected variants were annotated and filtered in Cartagenia Bench Lab NGS software using different criteria. The detected variants were scrutinized for their pathogenicity using Alamut Visual (Interactive Biosoftware).

Results: Nine different deleterious germline PV/likely pathogenic variants were identified in seven genes in 12 patients: three in *ATM*, and one in *CHEK2, ERCC5*, *FANCM*, *RAD51C*, *TP53* and *WRN*. Six of the 12 patients were carriers of a variant of uncertain significance (VUSs) in other genes. Several different VUSs were identified requiring further characterization.

Conclusion: In order to improve the clinical follow-up, more knowledge is needed regarding the diversity of genetic risk factors possibly involved in cancer development. Currently, for carriers of PV/LPV in many of these genes, there are no clinical management programs in Norway.

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P12.027D
Molecular characterization of tumour DNA from patients with non-small cell lung cancer

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Introduction: Cancer is often characterized as an evolutionary process driven by sequential acquisition of somatic mutations and clonal selection. Therefore, genomic analysis of cancer cells has greatly enhanced our ability to identify genetic alterations associated with various cancer types. Taking this in mind, cancer diagnosis and treatment is undergoing a fundamental shift from a histopathologically defined tumour treated with chemotherapy towards molecular characterisation of the tumour and use of targeted therapies. The challenge of clinical centres is to meet the needs for molecular testing using reliable methods and processes to ensure the patient receives accurate results in a timely manner. This study is focusing on the identification of actionable mutations in non-small cell lung cancer (NSCLC) patients that could inform the clinician in regards to diagnosis, prognosis and therapy decision.

Materials and Methods: Formalin-fixed, paraffin-embedded (FFPE) tumour DNA from 57 patients diagnosed with NSCLC were subjected to NIPD Genetics proprietary targeted capture enrichment technology followed by NGS. Sequencing data were validated by an independent laboratory using Ion Torrent platform and Ampliseq colon and lung cancer panel from Thermofisher.

Results: The most frequent mutations were identified in TP53 (46.81%), KRAS (14.89%), EGFR (10.64%) and PIK3CA (10.64%). Results were validated with IonTorrent-NGS for 26 patients for which there was adequate DNA material from the same tissue sections with a 97.3% concordance between the two methods.

Conclusion: These results highlight the clinical utility of our assay as a companion diagnostics tool that can inform cancer diagnosis, prognosis and guide therapy.

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P12.028A

Interventions in a Specialist Drug Development Unit to improve family history documentation and onward referral of patients with advanced cancer to Cancer Genetics Services

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Molecular aberrations in cancer may represent therapeutic targets, and, if arising from the germline, impact further cancer risk management in patients and their blood relatives. Annually, 600-700 patients are referred for consideration of experimental drug trials in the Drug Development Unit (DDU) in our institution. A proportion of patients may merit germline genetic testing because of suspicious personal/family history or findings of tumour-based testing. We aimed to assess the impact of different multi-disciplinary interventions on family history taking in and referral rates from DDU to Cancer Genetics Unit (CGU).

Methods:

Over 42 months, three interventions were undertaken at different intervals;

1. Embedding a genetics provider in DDU review clinic
2. "Traffic light" system flagging cancers with heritable component
3. Virtual multi-disciplinary meeting (MDM).

Comparative analyses between intervals were undertaken, including referral rates to CGU, investigations and patient outcomes. Family history-taking in a sample of 20 patients managed in each interval was assessed by retrospective chart review.

Results: Frequency of family history taking, and referral to CGU, increased with each intervention, particularly, the virtual MDM (40%-v- 85%). Referral rates increased over the study period, from 0.1 referral/week (5/year, 0.36% total referrals) to 1.2/week (projected 63/year (3.81%). Forty-four (52%) patients referred required germline testing, in three of whom variants were identified. Non-attendance rates were low (6, 7%).

Conclusion: Patients in the DDU are unique, with long cancer histories and short estimated life expectancy. Multidisciplinary working between CGU and DDU facilitates germline testing of those patients that may otherwise miss the opportunity.

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P12.029B
Alterations of DNA Damage Response Pathway in Lithuanian Patients with Castration-Resistant Prostate Cancer

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Introduction: Prostate cancer (PCa) is a heterogeneous and dynamic disease, which usually progresses to castration resistant PCa (CRPC) - the highly lethal form. Recent data suggest that CRPC may carry the germline or sporadic mutations in DNA damage response (DDR) pathway genes and this cancer is commonly identified in families with a high predisposition to the breast and ovarian cancer. Next generation sequencing based-studies can uncover the complex genomic landscape of CRPC and identify novel targets for efficient anticancer therapies. The aim of our study was the assessment of DDR pathway alterations in Lithuanian cohort of CRPC cases using qPCR and NGS targeted gene panel.

Materials and Methods: DNA was extracted from leukocytes of 143 CRPC patients that enrolled the study during 2016-2018. Dominant germline gene mutations in peripheral blood leukocytes were analyzed by custom made TaqMan assays and by NGS using custom made panel.

Results: DNA mutation in at least one of the five DDR genes was detected in 23 of 143 (16%) CRPC cases: in *BRCA1*, *BRCA2*, *CHEK2*, *NBN* and *ATM* were detected 2 (1,4%), 3 (2,1%), 12 (8,4%), 1 (0,7%) and 5 (3,5%), respectively. The most predominant mutation of the DDR pathway was the *CHEK2* c.470T>C mutation.

Conclusions: Germline or sporadic mutations of DDR pathway genes were detected in 16% of CRPC cases showing significant involvement of this molecular pathway in progression of PCa to the most aggressive form of the disease. In the future, specific treatment regimens should be considered for this subgroup of CRPC cases.

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P12.030C
Expression of *CD24* in plasma, exosome and ovarian tissue samples of serous ovarian cancer patients

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CD24 is a small molecular weight cell-surface protein and an independent marker for poor prognosis in the different type of cancers. Determination of CD24 expression in plasma, exosomes and ovarian tissue samples of serous ovarian cancer patients was our aim.

Ovarian tissue and blood samples from 21 cases of serous ovarian cancer and eight healthy controls were collected. After RNA isolation, the cDNA was synthesized, and then quantitative real-time PCR was performed using beta-globin as a housekeeping gene for the normalization of the data. Protein-protein and miRNA network analysis were performed using Biogrid and miRTargetLink databases.

Significant difference in the expression of CD24 in ovarian tissue between controls and patients (0.1595 ±0.3213 vs. 44.97±68.06 p=0.0068) was observed, while CD24 expression was detected in exosomes in 38.1% of patients, mainly with FIGO III, and in plasma 9.5% of cases. Correlation in the expression of CD24 and FIGO stages was determined between controls and patients. Our network analysis shows LYN, SELP, FGR, and NPM1 proteins are interacting with CD24.

Our research work demonstrated higher expression of CD24 in ovarian cancer patients' tissue samples, and there was an association with FIGO classification. However, CD24 expression was detected only in some cell-free plasma and exosome samples.

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P12.031D
CDH1 pathogenic variants in patients who do not fulfill testing criteria are not necessarily incidental findings

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Introduction: Multigene panel testing may identify carriers of pathogenic/likely pathogenic variants (PV/LPV) in the *CDH1* gene without a personal and/or family history of diffuse gastric and/or lobular breast cancer. Clinical interpretation of such cases can be difficult.

Methods: We performed a retrospective analysis of all identified cases of germline PV/LPV in *CDH1* since the introduction of the Illumina's TruSight Cancer sequencing panel at our Institute.

Results: Multigene panel testing was performed in 2448 cases of suspected hereditary cancer predisposition. Heterozygous PV/LPV in the *CDH1* gene were identified in 8 unrelated probands. 4/8 cases fulfilled the International Gastric Cancer Linkage Consortium (IGCLC) 2015 criteria, one only after revision of the gastric tumor specimen. The remaining 4/8 cases failed to fulfill IGCLC criteria. In two of these, there was a verified family history of mucinous and mixed-cell gastric cancers unavailable for revision. In the two other cases, the probands were screened for hereditary breast-ovarian cancer with a panel which included *CDH1*. Of these, there was limited data regarding family history in one case, and an unverifiable family history of second-degree relatives with gastric cancer in the other. In our cohort, we identified 22 carriers of variants of uncertain significance (VUS) in *CHD1*, most with a low likelihood of pathogenicity.

Conclusions: Testing for *CDH1* variants in patients who do not fulfill the IGCLC criteria resulted in a small number of identified PV/LPV carriers. Due to incomplete family histories, these cannot be regarded as true incidental findings. The VUS detection rate was low.

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P12.032A
Evidence for the association of the c.146T>C variant in *CDKN2A* with hereditary pancreatic ductal adenocarcinoma and melanoma syndrome in the Latino population

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Germline mutations in the *CDKN2A* gene cause a hereditary pancreatic ductal adenocarcinoma (PDAC) and melanoma syndrome. The presence of a *CDKN2A* pathogenic variant has a significant impact in determining appropriate screenings in carriers. Thus, accurate variant interpretation for *CDKN2A* gene alterations is of utmost importance. There are conflicting reports of pathogenicity for a specific variant (c.146T>C) in this gene. This variant has been classified as a non-actionable variant of uncertain significance by four laboratories while one laboratory has classified it as likely pathogenic and thus clinically actionable. Of interest, this variant is present in the ExAC database at a high frequency in the Latino population and, based on clinical data provided to laboratories, an association with this variant and PDAC in this population is postulated. We present four pedigrees with Latino ancestry and the *CDKN2A* c.146T>C variant. The pedigrees are consistent with a hereditary PDAC and melanoma syndrome. In two families, the mutation was documented in probands with a personal and family history of PDAC. The third proband has a personal history of melanoma and four first-degree relatives and two second-degree relatives with PDAC. The fourth proband is unaffected with a second-degree relative with PDAC. These clinical histories will assist with defining the pathogenicity of this variant. It is documented that surveillance is more successful at detecting PDAC at a resectable stage in *CDKN2A* mutation carriers and thus accurate classification of likely pathogenic or pathogenic variants plays an important role in determining appropriate surveillance in mutation carriers.

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P12.033B
A large intergenic deletion upstream of *CDKN2A* predisposes melanoma

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Introduction: Mutations in the *CDKN2A* gene that affect function of the p16INK4a transcript are the most common cause of familial melanoma. With the advent of next-generation sequencing a few more susceptibility genes have been identified over the last decade. Yet, approximately 50% of familial melanoma cases have no known attributed genetic cause, despite suggested autosomal dominant inheritance of disease.

Materials and Method: Whole genome (WGS) or exome (WES) sequencing was performed on DNA samples from 529 individuals in 267 high-risk families with cutaneous or uveal melanoma.

Results: WGS identified a 234 kb deletion approximately 200 kb upstream of the *CDKN2A* gene in 20/23 affected individuals from a large cutaneous melanoma family. In the neighbouring gene *DMRTA1*, we identified a haplotype-linked rare (gnomAD variant allele frequency = 8×10^{-5}) coding variant. Screening of melanoma families worldwide identified a further eight families harbouring this deletion.

Genetic ancestry analysis in combination with detailed examination of records revealed a shared ancestor for three of these families, though likely all originated from a common founder. Functional assessment of how this deletion modifies risk of cutaneous melanoma is on-going.

Conclusions: The deletion upstream of *CDKN2A* is the most common mutation to have been found in cutaneous melanoma families. This finding is of further significance due to the distance of the deletion from *CDKN2A* and that it would not be detected using traditional exome screening.

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P12.034C
EGFR mutation testing in cf-DNA from NSCLC patients using Cobas® EGFR mutation test v2

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Introduction: EGFR mutation analysis in circulating cell-free tumor DNA (cf-DNA) from plasma constitutes a less invasive alternative method in cases without adequate tumor material for molecular analysis and for monitoring disease progression.

Materials and Methods: 275 blood samples from NSCLC patients (73 with matched paraffin-embedded tumor tissues) were examined for EGFR mutations. Circulating cell- free tumor DNA (cf-DNA) isolation from plasma and detection of EGFR mutations was performed using Cobas® EGFR mutation test v2.

Results: EGFR mutations in cf-DNA were found in 22% of the samples. The most common EGFR mutations were exon 19 deletions (65%), followed by exon 21 point mutations (30%). The presence of mutations in primary NSCLC reached 12% whereas the rate of mutations was 33% for NSCLC patients during follow up. A resistance mutation, p.Thr790Met was displayed in 27% of the mutant cases. The analysis of 73 plasma cf-DNA samples versus matched tumor DNA showed 82% concordance with 62% sensitivity, 100% specificity, 100% PPV, 75% NPV, for the detection of EGFR mutations.

Conclusions: We observed a very high Positive Predictive Value of cf-DNA testing using Cobas® EGFR mutation test v2 indicating that EGFR mutations could be reported with certainty, whereas for patients with an EGFR mutation-negative cfDNA test, a biopsy should be obtained in order to ascertain an accurate result, according to current guidelines. In addition, as the resistance mutation p. Cys797Ser can not be evaluated by this method further testing is needed in this regard.

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P12.035D
Screening for germline *CHEK2* mutations in Spanish families with hereditary cancer suspicion

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Introduction: *CHEK2* pathogenic variants have been associated with increased risk of different types of cancer, mainly breast cancer. The aim of this work was to study *CHEK2* mutations in hereditary cancer patients from Spain. In a first step, we analyzed the three most prevalent *CHEK2* mutations in a hereditary breast and ovarian cancer cohort (HBOC). Next, we extended the analysis to the entire gene to a larger hereditary cancer cohort.

Patients and Methods: Testing for recurrent *CHEK2* mutations was performed by MLPA and CSCE in 432 patients from a retrospective HBOC cohort, negative for *BRCA* mutations. Targeted NGS was used to screen for *CHEK2* point mutations and large rearrangements in a prospective cohort consisting in 1926 patients with hereditary cancer suspicion.

Results: In total we found 16 different mutations in 24 index cases (~1%); only one recurrent mutation, c.1100delC, was found in one case. Seventeen of these patients (71%) had breast cancer while the remaining seven were affected by other cancers (kidney, colorectal, ovarian, pancreatic and testicular cancers). Two of them were carriers of biallelic mutations, showing a severe phenotype.

Conclusions: Most of the *CHEK2* identified mutations are not the common european recurrent mutations and are present in patients outside the classical HBOC syndrome.

Further multicentric studies are needed to truly assess the role of this gene in the pathogenesis of the different hereditary cancer syndromes as well as to establish the associated risks and clinical management of the patients and their relatives.

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P12.036A

Chromosomal changes in chordomas - pilot study

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Introduction: Chordomas are very rare and slowly growing malignant bone tumors originating from remnants of the notochord. Although the morphology and immunological profile of the chordoma cells are well described the molecular and genetic mechanisms responsible for the chordomas development have not been sufficiently elucidated yet.

Material and Methods: Total 45 FFPE chordoma samples from 20 patients from 2004-2017 were included in the project. DNA isolation and Whole Genome Amplification (WGA) using RepliG (QIAGEN) and Picoplex (Rubicon Genomics) were performed (in every sample). Determination of chromosomal changes was performed using microarray technology (Sure Print G3 Unrestricted CGH (8x60K), Agilent) and massive parallel sequencing (VeriSeq, lllumina).

Results: DNA samples were of low quality and concentration. Even after RepliG WGA, hybridization on chip was not successful. After Picoplex WGA, chromosomal changes were successfully determined using the VeriSeq kit in only 26 samples (from 17 patients). Recurrently found changes were 1p loss, 1q gain, chromosome 7 gain, chromosome 14 and 18 losses. The found changes were confirmed using FISH. The resulting profiles did not change during the patients` disease progress.

Conclusions: Our pilot study revealed that microarray is inappropriate method for studying chromosomal changes in FFPE chordoma samples. The chromosomal changes were detected in 85% of patients tested by VeriSeq kit. Our findings corellate with published results, but clinical utility of these findings has to be evaluated.

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P12.037B

Homozygosity for the *CHEK2* p.Gly167Arg variant results in a unique cancer syndrome with multiple complex chromosomal translocations in peripheral blood karyotype

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Introduction: Chromosomal instability is a well-established cancer characteristic, which is rarely observed in healthy tissues. Mutations in DNA repair genes disrupt proper cell maintenance, causing structural or copy-number aberrations that lead to cancer susceptibility. Here we describe two reportedly unrelated patients that presented with unique cancer syndromes and an abnormal karyotype in peripheral blood lymphocytes, caused by homozygosity for the p.Gly167Arg mutation in *CHEK2*.

Materials and Methods: Patient 1 was genetically diagnosed by the TruSight One (TSO) panel and whole-exome sequencing (WES); Patient 2 was diagnosed by FoundationOne® Heme genomic analysis. Candidate variant confirmation and segregation analyses were done with Sanger sequencing. Karyotype analysis was performed on peripheral blood, bone marrow and other available tissues.

Results: Patient 1 presented with multi-organ tumorogenesis, while Patient 2 had early-onset acute myeloid leukemia. Both patients were found homozygous for the *CHEK2* c.499G>A; p.Gly167Arg variant. Karyotype analysis of peripheral blood lymphocytes revealed 30-60% multiple different chromosomal translocations. This karyotype was not observed in other tested tissues, including bone marrow, nor in another cancer patient with a different homozygous missense mutation in *CHEK2* (p.Ser428Phe).

Conclusions: CHEK2 is a tumor suppressor gene, encoding the serine-threonine kinase CHK2. Our findings support the role of CHK2 in DNA-repair, as highlighted by the multiple chromosomal translocations observed in lymphocytes from both patients. We suggest that homozygosity for p.Gly167Arg impairs the correction of DNA breaks, leading to increased susceptibility to multiple tumorigenesis or early-onset disease.

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P12.038C
Functional validation and characterization of non-coding regulatory drivers in Chronic Lymphocytic Leukemia

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The role of coding mutations in cancer development has been extensively studied. However, the identification of the non-coding regulatory regions and the genes mediating their effects, remains poorly understood. In a previous study (BioRxiv 174219), we identified 16 local modules of coordinated non-coding regulatory elements of the genome (cis regulatory domains or CRDs, FDR 5%) that accumulated an excess of somatic mutations in 150 patients with chronic lymphocytic leukemia (CLL), a cancer affecting B-cells. Of those regions, 15 were associated to 44 genes, candidates drivers of tumorigenesis.

In this work, we present the development of an *in vitro* cancer-like model in which transformed B-cells are evaluated for their ability to reproduce cancer phenotypes such as cell migration, proliferation and apoptosis. For 21 genes with increased expression due to hypermutated CRDs, we setup a transfection assay to overexpress the genes and evaluate the phenotypic changes. For 14 genes with observed decreased expression, a microRNA interference approach was designed to express artificial miRNAs with high target cleavage.

Challenges and limitations of this approach are the low percentage of B-cells in blood and subject to donor-dependent variability (3-15% of total PBMCs), and the difficulty of B-cell transfection and culture.

The identification of changes in transformed B-cells towards a cancer-like phenotype due to the expression changes of candidate genes will provide a functional validation for the computational approach used to identify hypermutated CRDs and it will potentially identify new genes driving tumorigenesis in CLL. Experiments are in process and first results will be presented.

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P12.039D
Identification of novel chronic lymphocytic leukemia subtypes using pathway mutation scores and consensus clustering

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Introduction: Chronic lymphocytic leukemia (CLL) is the most common adult leukemia with variable clinical course underlain by striking genetic heterogeneity. CLL features a handful of putative driver genes and, more interestingly, a large number of non-recurrently mutated genes with elusive clinical implications. The aim of this study was to unravel the prognostic impact of pathway somatic mutation patterns in CLL.

Materials and Methods: We focused on a cohort of 316 CLL patients defined by mutated IGHV with somatic mutation data gathered by International Cancer Genome Consortium. In this cohort we collected 4739 genes affected by nonsynonymous point and/or frameshift mutations. We performed gene set enrichment analysis to identify affected

biological pathways, applied set theory to reduce redundancy in enriched gene sets, calculated pathway mutation scores for each patient and performed consensus clustering. Finally, we evaluated the difference in time to therapy (TTT) between identified CLL clusters.

Results: We identified eight clusters differing in TTT ($p<0.0001$); seven of these were characterized by distinct affected biological processes: namely, cell adhesion (21/23 mutated cases in the cluster), membrane depolarization (27/32), synapse organization (18/22), glycosylation (19/25), Rho protein signal transduction (27/37), oxytocin signalling and/or renin secretion (26/40), and transport through ABC transporters (11/20; patients with the earliest need for therapy).

Conclusion: Among CLL patients with mutated IGHV we identified distinct subgroups with non-recurrently mutated genes involved in a limited number of biological pathways. Our findings have far-reaching implications for CLL diagnostics. Supported by AZV-MZCR 16-34272A, 16-29447A, CEITEC2020 LQ1601, MZCR-RVO 65269705, MUNI/A/1105/2018.

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The results of the search for markers of the TKI efficiency in patients with newly diagnosed CML using molecular genetic methods

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Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by pathogenic activity of the chimeric tyrosine-kinase BCR/ABL which stimulates uncontrolled cell proliferation. Primary resistance to the targeted tyrosine-kinase inhibitors (TKI) is registered in up to 20% of patients. The aim of the work is to search for possible markers of the efficacy of TKI therapy. We sequenced 60 exomes of patients with CML with different response to treatment on the platform NextSeq™ 550, Illumina. The microRNA expression profile ($n = 6$ with the optimal response, $n = 6$ with failure of therapy) was examined using Nanostring technology and nCountermiRNA Expression Assay. On an average, we found 280000 variants, which are currently under analysis. GWAS analysis using Hail did not reveal polymorphisms associated with the response to therapy. Based on the results of the expression analysis of 17 microRNAs with differential expression in two groups were selected ($p < 0.05$). The greatest difference in expression is shown for hsa-miR-4286. Analysis of the functional role of microRNA revealed 40 pathways enriched with genes regulated by 17 microRNAs. The most important pathway is Pathways in cancer (hsa05200, $p = 9.5 * 10^{-14}$). The pathways Chronic myeloid leukemia (hsa05220, $p = 0.00018$), and Acute Myeloid Leukemia (hsa05221; $p = 0.04$) are also enriched. Deeper data analysis will help to find the possible predictors of the response to therapy, as well as allow us to study the mechanisms of disease progression, and microRNAs may be prognostic markers of a response to targeted CML therapy.

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P12.042C
Noninvasive detection of cancer specific genomic changes in patients with colorectal cancer

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Introduction: Genomic analysis of circulating DNA extracted from plasma of cancer patients has shown potential to be used as noninvasive biomarker usable in detection of cancer specific genomic changes. Aim of the study was to test the applicability of combined approach based on parallel detection of CNVs and SNVs as noninvasively obtained biomarkers in cohort of patients with colorectal cancer (CRC).

Material and Methods: Circulating DNA of patients with clinical diagnosis of CRC extracted from plasma collected before and after surgical removal of primary tumor were analyzed using low coverage whole genome scan for detection of CNVs on whole genome level and high coverage targeted resequencing of CRC specific gene panel. For CNVs and SNVs specific data analysis homemade and commercially available bioinformatic tools were used.

Results: Cancer specific genomic changes resulting in decrease of CNVs signal detected over the whole genome were present in 42% and decrease of allelic ratio of cancer

specific SNVs in 30% of CRC patients after primary tumor surgery. If both types of genomic changes were analyzed in combination the detection rate raised to 50%. Most frequently mutated genes showing hypothesized SNVs specific signal decrease were *TP53*, *APC*, *PIK3CA*, *BRAF* and *KRAS*.

Conclusions: In half of patients cancer specific genomic changes represented by CNVs and SNVs shown hypothesized decrease after tumor removal. Such results could be potentially used in development of portfolio of non-invasively utilizable biomarkers usable in disease monitoring. Acknowledgements: This project was supported by Slovak Research and Development Agency under the code APVV-14-0273.

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P12.043D
CMMRD-diagnostics and verification of homozygous variants in *PMS2*

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Background: Constitutional Mismatch Repair Deficiency (CMMRD) is a hereditary cancer predisposition for hematological, brain, and other tumors starting from childhood caused by biallelic variants in *MLH1, MSH2, MSH6, PMS2*.

Patients and Methods: We examined three patients (P1, P2, P3) with clinical indication of CMMRD carrying homozygous *PMS2* variants, whose pathogenicity and biallelic state should be verified, as *PMS2*-analysis is error-prone due to the presence of multiple pseudogenes.

RNA was isolated from blood-lymphocytes cultured for short-term in absence/presence of puromycin (cDNA-P/+P) to investigate transcripts with active/inhibited nonsense-mediated mRNA decay (NMD). From cDNA, the complete *PMS2* transcript was amplified and sequenced for variants or isoforms.

Results: A homozyogous exon 12 duplication was detected in P1. cDNA-analysis for her and her parents showed, that the duplicated exon was inserted in the transcript (r.2007_2174dup p.Lys670_Ala725dup). No wildtype allele was detected in P1, while present in both parents with 50% intensity indicating a heterozygous state.

The effect of an SINE-VNTR-Alu-insertion in intron 7 upon splicing was tested in P2. In cDNA+P, the insertion r.803_804insAATGTGCCATGTGAACCACCCCGTCTG AAAAGTGAGGAGCCCCTCTGCCCGGCAGCCGCCC CGTCTGGGAG p.Tyr268* was present in absence of the wildtype. In cDNA-P, the amplification failed, indicating that both mutated transcripts were subjected to NMD.

For P3 with the homozygous variant c.736_741delins TGTGTGTGAAG p.Pro246Cysfs*3, no cDNA was available. However, MLPA-analysis including a probe hybridizing to this region showed a complete dropout indicating a homozygous state of the variant in exon 7, for which no homologous pseudogene regions exist.

Conclusion: We confirmed the presence of homozygous pathogenic *PMS2* variants in three CMMRD-patients using cDNA-analysis or MLPA.

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P12.044A
Seven European colorectal cancer predisposing SNPs are associated with CRC and its prognosis in Turkish population

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Introduction: Colorectal cancer (CRC) is one of the leading causes of cancer-related death in developed countries. Owing to difficulty of the low allele frequency variations detection genetic association profile of CRC has not been entirely identified. To show that significance of these variations may change according to population we selected 16 SNPs from GWASs (Genome-wide Association Study) conducted in European CRC population to analyze them in Turkish population.

Material and Methods: Validation of 16 European SNP was performed using kompetitive allele spesific PCR on 1019 sporadic CRC cases and 948 controls. SNPs were excluded if their minor allele frequency was <5%, if the p-value of Hardy-Weinberg equilibrium test was $<10^{-6}$, or if the linkage disequilibrium $r^2>0.8$. Multiple logistic regression was used for additive, dominant, and recessive models. Odds ratios were adjusted for gender and the first principal component for population stratification (genomic control inflation factor $\lambda=0.903$). Further analysis is covered to classify the patients by their status of metastasis, cancer type, stage, grade, and tumor location.

Results and Conclusions: rs6983267, rs10795668, rs3802842, rs4444235 and rs4939827 were found to be associated with Turkish CRC patients ($p<0.05$). Our results showed that these 5 SNPs were also associated with pathological stage of the disease. Whereas, rs6691170, rs1321311, rs3802842, showed significant association with pathological grade. In addition, rs1321311 increases the risk of mucinous cancer 3,2 times. Our results showed the importance of population specific studies to validate the results of GWASs. This study is supported by TUBITAK grant 112S634, Ankara University 14L0415003

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P12.045B
A blood-based gene expression signature for diagnosis and prognosis of colorectal cancer using integrated genomic and network analyses

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Introduction: Colorectal cancer (CRC) is the third most common cancer and second leading cause of cancer-associated death worldwide. Diagnosing CRC patients reliably at an early and curable stage is of utmost importance to reduce the risk of mortality.

Materials and Methods: We identified a blood-based gene signature by performing an integrated genomic analysis of whole-genome gene expression and copy number alteration of Saudi CRC patients. The identified gene signature was then validated using independent datasets of gene expression profiling of CRC patients. In addition, we validated the gene signature's classification performance and the prognostic potential using TCGA (microarray and RNAseq) datasets with samples from over 800 CRC patients with detailed clinical data. We also identified the significantly altered pathways in CRC.

Results: The results have demonstrated that high expression of our gene signature is significantly associated with poor disease outcome. The pathway analysis revealed alterations in various cancer-related pathways essential for CRC transformation; hence validating our gene signature's diagnostic and prognostic potential using patients' biological fluids rather than invasive procedures.

Conclusions: Results suggest that integrated genomic analysis may provide a reliable approach to identify key biological programs associated with CRC and lead to improved diagnosis and therapeutic options. Funding: This study is funded by the KFSHRC Research Grant (2110006 to DC).

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P12.046C
The frequency of *MLH1* and *MSH2* germline mutations in colorectal cancer patients met the revised Bethesda Guidelines

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Introduction: Lynch syndrome (LS) is one of the most common hereditary cancer syndromes that can be identified in 3%-5% of all colorectal cancer (CRC) patients. To identify individuals with high risk of LS the Amsterdam criteria (AC) and the Bethesda Guidelines (BG) have been proposed. This study aimed to analyze the frequency of *MLH1* and *MSH2* germline mutations in CRC patients met the revised BG.

Materials and Methods: One hundred and seventeen patients fulfilling the revised BG were initially screened for LS by Sanger sequencing of the coding regions and splice sites of the *MLH1* and *MSH2* genes. MSI-diagnostics and *BRAF*-genotyping were performed in 95 of 117 tumour samples. Clinical, anamnestic and morphological characteristics were recorded. The accuracy of the AC was evaluated.

Results: We identified 29 patients with germline mutations in the *MLH1* and *MSH2* genes (17 and 12 patients, respectively). The detected variants were found throughout the coding part of the genes. Nine *MLH1*-mutations have been described for the first time. The highest accuracy was

shown for the ACI (85%). High-level MSI and no *BRAF*-mutation were found in all analyzed LS-tumours.

Conclusions: The results of our study prove that MSI approach provides a sensitivity of 100% for identifying LS-patients. The ACII missed as many as 31% of LS-cases. Being quite stringent the ACI showed a high rate of specificity but failed to recognize 41% of LS-patients.

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P12.047D
K-ras mutations and *MGMT* methylation in CRC and correlation with survival and therapy

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Introduction: Colorectal cancer (CRC) is one of the most frequent cancers in humans. The disease occurs sporadically in most of the cases (75%-80%) as a result of the accumulation of both mutations and epigenetic modifications of several genes. Approximately 20-50% of primary colorectal tumors contain *K-ras* mutations and it was shown that *MGMT* methylation may play a role preceding the development of CRC.

Materials and Methods: Two hundred and forty-nine fresh colorectal tumor samples and corresponding normal tissue were collected during surgical colectomy together with clinical and pathological data. Patients were followed up for ten years. Direct sequencing was used for detection of *k-ras* mutations and methylation-specific PCR was performed to assess the methylation status of *MGMT*.

Results: *K-ras* mutations were detected in 83/249 (33%) and hyper-methylated promoter of *MGMT* in 220/249 (88%) tumors. Survival distribution of patients with and without *K-ras* mutation was significantly different (p=0,01). Patients with metastasis and hyper-methylated *MGMT* promoter in their primary CRCs had significantly worse survival distribution than patients without hyper-methylation (p=0,05), while in non-metastatic cancers this was not the case. Response to radiation therapy was significantly better in tumors with methylated *MGMT* promoter (P< 0,001).

Conclusion: No significant correlation was observed between *K-ras* mutations and *MGMT* methylation. *MGMT* promoter methylation might be a good diagnostic marker in normal-appearing mucosa, both for early detection of and risk assessment in colon cancer, as well as may predict a better response to radiation therapy, as we have shown in this study.

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P12.048A
Variant detection using transcriptome analyses in hereditary colorectal cancer

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Introduction: High-penetrant pathogenic variants in established genes associated with colorectal cancer (CRC) explain the disease in approximately 5-6% of cases. However, a genetic component is suspected to contribute to CRC in approximately 20%-30% of all cases. Transcriptome analysis reveals established pathogenic variants as well as alternative mechanisms affecting transcription in candidate genes.

Material and Methods: Transcriptome analyses, (Illumina paired-end read sequencing (2 x 150 bp)), were performed on 50 patients without any causative variant detected with commonly used techniques. In parallel the patients were analyzed on a comprehensive panel including high-penetrant susceptibility genes and candidate genes (50 kb upstream and downstream UTR regions, intronic and exonic regions). The patients in the study were referred to the Cancer Genetic Counselling Clinic at Sahlgrenska University Hospital, Gothenburg, Sweden.

Results: Analysis of alternative splicing, identification and quantification of transcripts, classification of SNVs and CNVs were performed and findings were thoroughly evaluated concerning pathogenicity. Expression level comparisons were executed both pairwise and against positive controls with known causative variants. The findings include identification of previously unreported variants and putative causative variants in patients with previously unexplained hereditary CRC.

Conclusion: The combination of high-throughput NGS-techniques on genome and transcriptome level identifies a

broader set of pathogenic variants in patients with hereditary CRC, which is crucial for follow up of patients.

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P12.049B
Exploring the role of *CCAT1* and *CCAT2* long non-coding RNA in colorectal carcinoma metastasis

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Introduction: Dysregulation of long non-coding RNAs (lncRNAs) has been recently implicated in cancers. Up-regulation of two lncRNAs, *CCAT1* and *CCAT2* has been separately associated with more aggressive colorectal cancer (CRC) and worse prognosis. The first GWAS-identified SNP linked to CRC risk, rs6983267, maps to *CCAT2*, and is reported to elevate the expression of *c-Myc*, situated about 300 kb away. Nevertheless, these two lncRNAs have never been studied together with their oncotarget c-Myc.

Material and Methods: In this study, we measured the expression of *CCAT1*, *CCAT2* and *c-Myc* in 150 matched mucosa-tumor samples of early stage microsatellite-stable Chinese CRC patients with definitive metastasis status by real-time RT-PCR. The expression profile is then correlated to the SNP genotype and the clinical data.

Results: Expression of *c-Myc* in the tumor is significantly up-regulated in metastasis-positive CRC patients (T-test, $p=0.0229$) and significantly correlated to time to metastasis by Cox regression analysis [Hazard Ratio = 1.47 (1.10, 1.97), $p=0.0107$]. Upregulation of *c-Myc* expression is also significantly associated with the risk genotype (GG) of rs6983267. The expression of *c-Myc* is significantly ($p<0.0001$) correlated to that of *CCAT1* and *CCAT2* ($R^2=0.23$ and $R^2=0.18$ respectively) in the tumors. However, the expression of *CCAT1* and *CCAT2* is not significantly correlated to metastasis status, time to metastasis or the SNP genotypes.

Conclusions: Expression of *c-Myc* in the tumors is predictive of metastasis. The expression of *CCAT1* and *CCAT2* does not add to the predictive value suggesting that their contribution to metastasis are probably accounted for via their effects on *c-Myc*.

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P12.050C
Ovarian clear cell carcinoma in Cowden Syndrome

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Purpose: Cowden Syndrome (CS) is an autosomal dominant disease related to *PTEN* germline mutations. CS is characterized by macrocephaly, mucocutaneous lesions and an increased risk of breast and thyroid cancer. Rare ovarian cancer cases (mostly embryonic tumours) associated to *PTEN* have been described in the literature and there are no current CS guidelines available for ovarian cancer risk management.

Clinical Presentation: We report the case of a woman diagnosed with ovarian clear cell carcinoma (OCCC) at age 28. The patient dysplayed macrocephaly, trichilemmomas, oral papillomatosis and acral keratosis. A thyroid follicular neoplasia was diagnosed synchronously. A family history of cancers within the *PTEN*-related tumor spectrum was identified. *PTEN* sequencing revealed that she carried a germline inherited pathogenic variant c.388C>T, p.(Arg130*) (NM_000314). A somatic MMR immunohistochemistry analysis showed a normal expression and *BRCA1/2*-germline sequencing revealed no mutation. An ovarian cell immunohistochemistry analysis reported total loss of PTEN expression, which strongly suggested the *PTEN* role in the oncogenesis of this cancer. Hence, a total thyroid resection was performed instead of thyroid lobectomy and bilateral mastectomy was discussed. Co-occurrence of this germline mutation in *PTEN* in this

patient, early development OCCC at age 28 and total loss of *PTEN* expression in the tumor support the involvement of *PTEN* in the cancerogenesis of her ovarian cancer.

Conclusion: We describe a new ovarian cancer case with an atypical histological type, clear cell carcinoma, in CS. This observation might be a first clue to expand PTEN-related tumor spectrum with OCCC. The CS diagnosis changed her management.

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P12.051D
Germline variants in oculocutaneous albinism genes in familial cutaneous melanoma

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Introduction: Oculocutaneous albinism (OCA) is a group of autosomal recessive genetic diseases, resulting in pigmentation defects of the eyes, hair and skin. OCA is caused by compound heterozygous or homozygous germline variants in one of four genes: *TYR, OCA2, TYRP1* and *SLC45A2*. Due to a lack of melanin production, OCA patients have a heightened sensitivity to ultra-violet radiation, predisposing them to skin cancers, including cutaneous melanoma (CM).

Methods: Approximately 5-12% of CM is considered familial. We sought to investigate the unexplained predisposition in families negative for the known susceptibility genes using next generation sequencing of affected individuals. Variants of interest were confirmed and cosegregation was assessed by Sanger sequencing.

Results: Several heterozygous germline variants in *TYR, OCA2, TYRP1* and *SLC45A2* were present in our familial CM cohort. Of note, are variants classified as pathogenic in association with OCA that were either present in multiple families or fully cosegregated with CM. The TYR p.T373K variant was present in 3 unrelated CM families. In OCA2, we observed p.V443I in three families and p.N489D in two families. We also identified a novel likely pathogenic *SLC45A2* frameshift variant fully co-segregating in a family of 4 CM cases.

Conclusions: Rare, heterozygous variants in OCA genes *TYR, OCA2, SLC45A2* and *TYRP1* occur in some familial CM cases. Cosegregation analysis showed similar results to the medium penetrance *MITF* p.E318K variant, and the known functional consequence of these albinism gene variants on pigmentation pathways indicates these OCA genes may increase CM susceptibility in some families.

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P12.052A
Evaluation of tamoxifen efficacy and*CYP2D6*-genotyping in women with hormone receptor-positive breast cancer

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Introduction: Tamoxifen is one of the most frequent prescribed drugs to treat patient with estrogen receptor positive breast cancer (BC). In vivo Tamoxifen is biotransformed by the CYP2D6 enzyme into endoxifen. The aim of the study was to evaluate the effectiveness of therapy with Tamoxifen in patients with luminal A and B primary operable BC by *CYP2D6*-genotyping.

Materials and Methods: One hundred and eleven women with IA-IIB stage-BC receiving tamoxifen were enrolled in the study. Real-time PCR for detection of allelic variants *CYP2D6*3* (c.2549delA), *CYP2D6*4* (c.1846G>A) and *CYP2D6*6* (.1707) was performed. Relapse-free survival (RFS) was estimated depending on the *CYP2D6*-genotype.

Results: We revealed 78 patients (70,2%) with wild-type alleles of the *CYP2D6* gene (extensive metabolizers(EM)), 26 women (23,4%) - intensive metabolizers (IM) and 7 patients (6,3%) – poor metabolizers (PM). The average duration of Tamoxifen-therapy was 2,7 years. We observed differences in RFS in patients treated only Tamoxifen in the adjuvant period. RFS in women with EM-phenotype was better than in IM, PM-patients (2,6% (2/78) vs 18,2% (6/33) ($p=0.02$)). We found no differences in RFS in patients receiving polychemotherapy in combination with hormone therapy ($p>0.05$).

Conclusion: Patients with estrogen receptor positive primary operable BC and reduced CYP2D6-activity

characterize by worse RFS. *CYP2D6*-genotyping is one of the instruments to predict the effectiveness of Tamoxifen-therapy.

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P12.053B
Correlation of mRNA-TSHR expression with clinical parameters in thyroid carcinoma patients

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Introduction: Differentiated thyroid carcinomas (DTC) preserve expression of thyroid stimulating hormone receptor (TSHR).

Aim: Objective in our study was to evaluate expression of mRNA-TSHR in patients with DTC and then to correlate the expression with clinical features: thyroglobulin (sTg) value, initial staging, whole body scan (WBS), neck ultrasound (US) and received dose of radioiodine.

Material and Methods: Forty patients were subdivided in groups according response to treatment: patients with incomplete structural response (TCs), incomplete biochemical response (TCb) and excellent responders (TCr). Total RNA was isolated from peripheral blood and used for two-step reverse transcriptase-polymerase chain reaction (rt PCR) with appropriate primers. Relative quantification with the $2^{-\Delta\Delta Ct}$ method was applied. Thyroglobulin was evaluated with immunohistochemical analysis. Initial stage according TNM staging system was recorded. Statistical analysis was performed with Spearman Rank Order Correlation.

Results: Patients from TCs group expressed mRNA-TSHR by a 5.37-fold higher level than TCr patients, TCb expressed TSHR by an 8.88-fold higher level than TCr patients. Significant negative correlation was detected between sTg and ΔCt (Ct_{TSHR}-Ct_{GAPDH}) value (R=-0,475; p<0,05) and between WBS/US findings and ΔCt (R= -0,321; p<0,05). Correlation between initial stage and ΔCt revealed non-significant negative correlation and non-significant positive correlation between received dose of radioiodine and ΔCt.

Conclusion: Our data revealed higher expression of mRNA-TSHR in blood of TCs and TCb compared to TCr group and further analysis revealed significant correlation between mRNA-TSHR and sTg and US/WBS findings. Further studies with absolute quantification are needed for understanding the real meaning of mRNA-TSHR as biomarker in DTC.

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P12.054C
Comparison of genomic profiles in patients with primary and recurrent diffuse brain gliomas revealed acquisition of biologically relevant genomic aberrations

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Diffuse gliomas represent heterogeneous group of brain tumors with highly variable biological behavior. Despite of radical surgical resection and intensive therapy, recurrences with new genetic properties frequently appear. We compared genomic profiles of 11 paired primary and recurrent gliomas to identify aberrations related to tumor recurrence and/or progression.

Tumor samples obtained during routine surgery were analyzed with SNP array (Illumina), I-FISH (Abbott, MetaSystems), MLPA (MRC-Holland) to assess copy number variations and *IDH1* mutations and with MS-MLPA to investigate methylation of *MGMT* and *MLH3* promoters.

In both primary and recurrent lesions, we observed genomic changes typical for gliomas: loss of chromosome 10, gains on chromosome 7, losses on 9p and 13q, 17p LOH and/or *IDH1* mutations. In 7/11 recurrences, the genetic profile showed additional chromosomal imbalances compared to primary tumor. In 4/11 pairs, the profiles were divergent, i.e. the recurrences included additional changes but missed some of the original ones. Losses of chromosomal regions 6p21.33, 6q27, 9q21.13, 11p11.2 and hypermethylation of *MGMT* and/or *MLH3* were repeatedly

detected in recurrences. The progression to a higher grade of glioma occurred in 5/11 patients.

We suppose that recurrence of brain gliomas may be driven by proliferation of cells with additional genomic imbalances to those detected in primaries or by one or more subclones presented within the primary tumor besides the major tumor clone. Our results indicate that recurrences are often genetically and/or epigenetically different from primary lesions, therefore the patients with recurrent tumors may benefit from different treatment approaches.

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P12.055D

Family History Questionnaire System (FHQS): An innovative patient facing online software application streamlining assessment of genetic susceptibility to cancer

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Screening, Prevention and Early Detection (SPED) in cancer management is cost effective and clinically preferable to other therapeutic interventions. SPED activities decrease the likelihood of an individual developing cancer, and enable an increased number of cancers to be detected at a curable stage. Identifying those who have an increased genetic susceptibility to developing cancer enables targeting of SPED interventions at those at highest risk and is the most clinically efficient and cost effective way of implementing SPED interventions. However, current methods of identifying genetically "at risk" individuals through family history assessment are inequitable, non-standardised, resource heavy and time consuming. The Cancer Genetics Unit of the South West Thames Regional Genetics Service (SWTRGS) at St George's Hospital (SGH) has developed the Family History Questionnaire System (FHQS). This is an online patient-facing cancer family history assessment software application to enable competent, standardised cancer family history assessment which is more efficient, cost effective, scalable and equitable than current methods. We will present data from the initial pilot phase and clinical implementation of this innovative app in the SWTRGS population of 3.4 million people regarding:

- Patient experience and acceptability and improved access to assessment for cancer patients and their relatives
- Development and implementation of novel and streamlined clinical pathways for assessment, access to SPED interventions and genetic testing
- Data privacy and regulatory considerations
- Data storage and integration with clinical systems and access to research

Our app demonstrates the power of digital innovation to improve clinical pathways for patients with inherited susceptibility to cancer.

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P12.056A

Exome sequencing identifies germline variants in *DIS3* in familial multiple myeloma

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Multiple myeloma (MM) is an incurable plasma cell malignancy. While the mutational landscape of sporadic MM has been extensively investigated, the genetic basis of familial MM remains largely unknown.

We used exome and targeted sequencing in families with several cases of MM/MGUS to identify rare germline variants implicated in MM susceptibility.

We observed putative loss-of-function (two splicing (c.1755+1G>T, c.1883+1G>C) and one stop-loss (c.2875T>C, p.*959Glnext*14)) germline variants in *DIS3*, a frequently somatically mutated gene in MM, in 4 out of 154 families (2.6%). We demonstrated that the c.1755+1G>T allele undergoes Nonsense-Mediated Decay (NMD), resulting in a marked reduction in *DIS3* gene expression levels, while the p.*959Glnext*14 variant reduces DIS3 protein levels, consistent with loss-of-function variants. In an independent series of 781 sporadic MM patients and 3534 controls from the MMRF CoMMpass Study we demonstrated that rare, putative likely deleterious variants in *DIS3* were enriched among MM patients compared to controls (OR= 1.92 95%CI:1.25 to 2.96, p=0.001). Transcriptome analysis of MM tumor cells suggested that, in comparison to non-carriers, carriers of germline *DIS3* likely deleterious variants showed dysregulation of RNA processing pathways, consistent with disruption of DIS3 function.

Our observations are consistent with a role for *DIS3* in genetic susceptibility to MM/MGUS and reinforce its role in myelomagenesis.

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P12.057B
High-throughput sorting of tumor cell populations reveals the composite mutational landscape of oesophageal adenocarcinoma (EAC)

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Esophageal adenocarcinoma (EAC) is characterized by a high genetic heterogeneity, which accounts for therapeutic resistance and tumor recurrence. Outcomes of most genomic studies have important limitations due to the presence of different cell types within these cancers. Our study aimed at investigating intra- and intertumor heterogeneity by targeted sequencing of sorted cancer subpopulations. Archival material from 38 EACs, classified according to Lauren, was processed using a high-throughput cell sorting technology. Stromal and tumor cell populations were sorted by immunolabelling for vimentin/pan-cytokeratin and DNA content. Targeted sequencing was performed for 63 cancer-related genes. 31 out of 38 EAC carried at least one somatic mutation, not present in the corresponding sorted stromal cells. In 25/38 (65.7 %) of cases mutations were detected in *TP53* and in 5/38 (13.2%) of cases in *CDKN2A*. Mutations in other genes occurred at lower frequency, including *HNF1A* (2/38 cases), not previously associated with EAC. Most of the mutations were also identified in unsorted cells, however with a lower allele frequency, whereas in one case the mutation was completely missed. Interestingly, in some EAC cases, we isolated different keratin-positive cell populations based on DNA content (hyperdiploid and pseudodiploid clones) with different mutational loads, confirming the high intra-tumor heterogeneity of these cancers. In conclusion, selective sorting allowed us to identify somatic mutations not identifiable in the unsorted cells and to isolate different cancer subpopulations, representative of different cancer subclones. Future studies will reveal the role of these subpopulations with respect to therapeutic response. FI is supported an ONCOPENTA fellowship.

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P12.058C
Germline loss-of-function variants in the *BARD1* gene are associated with early-onset familial breast cancer but not ovarian cancer

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The role of *BARD1* in breast cancer (BC) and ovarian cancer (OC) predisposition remains elusive, as published case-control investigations have revealed controversial results. We aimed to assess the role of deleterious *BARD1* germline variants in BC/OC predisposition. A total of 4,469 *BRCA1/2*-negative female index patients with BC, 451 *BRCA1/2*-negative index patients with OC, and 2,767 geographically-matched female controls were screened for loss-of-function (LoF) mutations and potentially damaging rare missense variants in *BARD1*. All patients met the inclusion criteria of the German Consortium for Hereditary Breast and Ovarian Cancer for germline testing and reported at least one relative with BC or OC. Additional control datasets (ExAC, FLOSSIES) were included for the calculation of odds ratios (ORs). We identified LoF variants in 23 of 4,469 BC index patients (0.51%) and in 36 of 37,265 controls (0.10%), resulting in an OR of 5.35 (95% confidence interval [CI] =3.17-9.04; P<0.00001). *BARD1*-mutated BC index patients showed a significantly younger mean age at first diagnosis (42.3 years, range: 24-60 years) compared with the overall study sample (48.6 years, range: 17-92 years; P=0.00347). Overall, rare and predicted damaging (SIFT, MutationTaster) *BARD1* missense variants were significantly more prevalent in BC index patients compared with controls (OR=2.15; 95% CI=1.26-3.67; P=0.00723). Neither LoF variants nor predicted damaging rare missense variants in *BARD1* were identified in 451 familial index patients with OC. Due to the significant association of germline LoF variants in *BARD1* with early-onset BC, we suggest that intensified surveillance programs should be considered for women carrying pathogenic *BARD1* gene variants.

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P12.059D

Evaluating the frequencies of *EGFR*, *ALK/EML4*, and *ROS1* alterations in lung cancer: A single-center experience

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Mutations in *EGFR* and rearrangements of *ALK* and *ROS-1* affecting Ras/Raf/MAPK signal transduction pathway provides prognostic importance since treatment strategies with a variety of tyrosine kinase inhibitors in lung cancer have been developed. Based on latest targetable drug therapies regarding these three genes, this study aimed at determining the mutation frequencies of them between 2015 and 2019. For *EGFR* mutations, tissue samples from 967 patients were analyzed by pyro-sequencing and Real-Time PCR while *ALK* rearrangements of 780 samples and *ROS1* of 437 samples were examined by FISH. However, 104 of 780 tissue samples could not be analyzed by FISH and detailed results are given in Table. In total, 17,27% of 967 samples showed mutation-positive profile for *EGFR* and 3,85% of 676 samples were determined as *ALK*-positive while 0,6% of 333 were *ROS1*-positive. In addition, T790M

Gene Name	*EGFR* (n=967)							*ALK/EML4* (n=676)					*ROS1* (n=333)	
Mutations	Positive (17,27 %)						Negative	Positive 3,85 % (n=26)				Negative	Positive	Negative
	E19	L858R	G719X	L861Q	S768I	T790M	82,73 % (n=800)	ALK +/EML4+	5'del ALK +	ALK +/EML4-	ALK-/ EML4+	96,15 % (n=650)	0,6 % (n=2)	99,4 % (n=331)
Number (n) & Frequency in %	4,75 %	3,41 %	3,21 %	2,38 %	2,38 %	1,14 %		n=16	n=5	n=4	n=1			

mutation known to induce secondary resistance was calculated as 6,5% of total *EGFR* mutations. Five of 26 *ALK*-positive patients show 5'deletion while four of 26 was determined as *EML4*-negative and the two others were also detected as having *EGFR* mutation L816Q. Moreover, *ALK*-negative and *EML4*-positive rearrangement was determined in a case. To conclude, both *ALK* and *EML4* positive cases should be assuredly examined together with respect to *EGFR* mutations like in our two cases because they are together known to cause decrease in efficacy of EGFR-TKIs in adenocarcinomas.

Table. Mutation frequency distributions over tissue samples.

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P12.062C

Multigene panel testing in breast/ovarian cancer patients reveals high prevalence of *FANCM* truncating variants and suggests an oligogenic disease course in the carriers

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Introduction: Recently, *FANCM* gene emerged as a novel moderate-risk gene for breast and ovarian cancer (BC/OC). Several studies have shown that *FANCM* truncating variants are particularly associated with ER- and triple negative (TN) BC. However, additional high, moderate and low-risk cancer genes in *FANCM* carriers have not been well studied. Here, we present the clinical and genetic data of BC/OC patients carrying truncating *FANCM* variants.

Material and Methods: We have sequenced 94 cancer-associated genes in 344 (331 BC, 10 OC and 3 BC/OC) patients using Illumina TruSight Cancer kit on a MiSeq platform.

Results: As expected, the highest prevalence of pathogenic variants was found in *BRCA1* (26 patients, 7.6%) and *BRCA2* (32 patients, 9.3%) genes. *CHEK2* and *FANCM* were the next most frequently mutated genes (5/344, 1.5% each). Four different truncating *FANCM* variants [c.1972C>T, p.(Arg658Ter), c.2953delG, p.(Glu958fs), c.5048_5022del, p.(Lys1683fs) and c.5791C>T, p.(Arg1931Ter)] were identified in three BC, one OC and one BC/OC patient. Interestingly, four (80%) of the *FANCM* carriers had co-inherited pathogenic variant in *BRCA2* (n=2), *BRCA1* (n=1) and *FANCE* (n=1) genes. These findings suggest that the penetrance of *FANCM* pathogenic variants depends on the co-inheritance of pathogenic variants in other cancer genes. The two *FANCM/BRCA2* carriers had ER+ BC, while *FANCM/FANCE* and only-*FANCM* BC carriers had TN BC, suggesting that the presence of *BRCA2* mutations modifies the BC ER expression in *FANCM* carriers.

Conclusion: Our study supports the finding of *FANCM* as a novel BC/OC susceptibility gene, and further suggests an oligogenic disease course in *FANCM* mutation carriers.

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P12.063D

Intermediate risk variant in *brca1* expands the clinical spectrum of fanconi anemia and sheds new light on variant classification criteria

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Heterozygous *BRCA1* mutations confer high life-time risks for breast and ovarian cancer, whereas biallelic *BRCA1* mutations lead either to embryonic lethality or Fanconi anemia (FA), a severe congenital syndrome mainly characterized by bone marrow failure, developmental abnormalities and solid tumors. By multigene panel analysis, we identified two compound heterozygous pathogenic *BRCA1* mutations, p.(Cys61Gly) and p.(Arg1699Gln), in a female index with breast cancer at the age of 30 years. The p.Cys61Gly mutation confers high cancer risk, while the hypomorphic p.Arg1699Gln mutation is associated with an intermediate cancer risk. A cytodiagnostic test for FA was negative in patient`s PBLs. Clinical investigations revealed mild FA-like features, i.e. low height (150 cm), mild microcephaly (52 cm circumference), café au lait macules, and severe chemotherapy-induced hematotoxicity. Functional analyses of the hypomorphic p.Arg1699Gln mutation revealed impaired BRCA1 functionality in DNA repair and replication forks stabilization. However, the residual activity of the hypomorphic p.Arg1699Gln allele likely prevents the severe FA phenotype. Our data expand the clinical spectrum associated with biallelic *BRCA1* mutations. Furthermore, these data may have implications for *BRCA1* variant classification using the co-occurrence model which is based on the assumption that pathogenic biallelic *BRCA1* mutations are lethal or cause severe FA.

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P12.064A
Functional analysis of driver mutations in the FGFR3 expanding with paternal age

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Introduction: Certain point mutations in the *FGFR3* gene have a very high mutation frequency in sperm compared to the genome average that increases with age. Studies proposed this effect to be linked with single nucleotide substitutions causing the expansion of spermatogonial stem cells following a germline selection model. As a tyrosine receptor kinase (RTK) family member, FGFR3 is involved in the regulation of cell proliferation, differentiation and apoptosis. Therefore, severe phenotypes are associated with aberrant receptor signaling caused by these mutations, which have also been detected in numerous human cancers.

Materials and Methods: We are currently screening *de novo* mutations in the *FGFR3* occurring at increased levels ($>10^{-5}$) in human sperm with duplex sequencing. In this study, we are testing the functional changes associated with the discovered mutations in a structure-function-analysis. Specifically, we are determining potentially altered signaling of different mutated FGFR3 in comparison to the wild-type receptor at three different levels: 1) Analysis of tyrosine phosphorylation in the kinase region of the receptor via phospho-specific Western blotting; 2) Measuring the dynamics of receptor stoichiometry on the cell surface using single molecule microscopy (TOCCSL); 3) Evaluation of intracellular calcium kinetics by ratiometric analysis of Fura-2 loaded cells.

Conclusions: In this study we are functionally characterizing newly described rare mutations arising in the male germline using highly quantitative biophysical methods. This study will provide new insights in the processes underlying increased signaling of mutated RTKs coupled with aberrant signaling in mutant cells.

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P12.065B
Molecular characterisation of various fusion transcripts in different histologic types of cancers

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Targeted next-generation sequencing is gradually being used in routine molecular pathology. Detection of gene fusions is useful for prognostics, and differential and predictive diagnostics. Here we review the results of fusion detection analyses in clinical routine samples including some retrospective studies collected over a two-year period. We present overall statistics and evaluate the suitability of the NGS approach compared to traditional methods and discuss the benefits and limitations of the analyses. Several Fusion Plex panels were employed focused on fusions found in solid tumors, sarcomas, lymphomas, and thyroid

and lung cancer. The kits use anchored multiplex PCR, an assay that targets only one partner of the fusion pair and allows to detect unknown fusion partners. Of 2505 analyzed samples, 348 were positive for some fusion transcript, 133 of those were unique fusion gene pairs, and out of that 58 were novel. Of the remaining 1771 samples were negative, 386 samples were not analyzable, mostly due to insufficient quality of input RNA. Novel fusion transcripts were confirmed either by FISH break-apart or fusion FISH probe or custom designed RT-PCR. The NGS method proved to be very efficient in detecting a large variety of fusion transcripts, the only notable limitation is the quality of source material.

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P12.066C
Genetic predisposition of familial Hdgkin lymphoma

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Hodgkin lymphoma (HL) is a B-cell lymphoproliferative neoplasms that affects 2.7 individuals per 100,000/yr. Risk factors include EBV infection, especially in the context of acquired or innate immunodeficiency. Despite evidence that genetic predisposition plays an important role in a subset of HL, there is a dearth of functionally validated disease-causative genes. We aim to identify germline genetic "drivers" of HL by applying Whole Exome Sequencing (WES) to rare, familial HL samples, followed by functional validation of prioritized candidate genes with rare, potentially pathogenic variants that co-segregate with disease within families.

A pilot study on 20 affected individuals from 10 families identified between 24 and 96 such altered genes per family, 23 of which were identified in more than one. Intriguingly, they include several proteins that regulate mitosis and mitotic checkpoints, suggesting this may be an important pathway in disease. Variants in the top two of these candidate genes -(i) one involved in the first step of glycolysis (the 2 identified variants result in decreased function) and (ii) the other a cell-cycle regulated E3-ubiquitin ligase- are currently being tested for their effects on protein function. We are further expanding the WES on an additional series of 18 families to confirm the recurrence of altered genes in familial HL and to look for somatic-second hit locally eliminating the wild-type allele, according to the classical paradigm for tumor-suppressor gene.

(Nisha Limaye and Hélène A. Poirel: Equal contribution)

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P12.067D
Temozolomide and AZD3463 have synergistic anticancer effect on T98G glioblastoma multiforme cells

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Glioblastoma multiforme (GBM) comprises most malignant phenotype of central nervous system tumors which are defined as grade IV. Temozolomide is an alkylating agent which is used for GBM treatment. Although temozolomide has a remarkable effect, the high application dose reveals the necessity of combination with other agents in the treatment of GBM. AZD3463 is a novel ALK tyrosine kinase receptor inhibitor which is over-expressed in GBM. The aim of this study was to investigate whether the inhibition of ALK positively affects the activity of temozolomide in GBM. Temozolomide and AZD3463 were dissolved in DMSO. T98G GBM cell line obtained from ATCC was cultured via EMEM. The cytotoxic effects of temozolomide, AZD3463 and their combination were determined in time and dose dependent manner by using WST8 (Biointersect) assay. IC50 values and the combination index (CI) were calculated by CalcuSyn (Biosoft). Effects of the agents on apoptosis and cell cycle status were determined by BD accuri C6 flow cytometry and Annexin V (BD Biosciences) and Cycletest plus DNA (BD Biosciences) assays, respectively. IC50 values of temozolomide and AZD3463 were determined as 1.54mM and 529nM on 48th hours, respectively. CI value was found 0.45 and the combination was evaluated as synergistic (ED75 value: 1.4mM temozolomide, 1.4μM AZD3463). Temozolomide, AZD3463 and the combination induced apoptosis 2.76, 1.17, and 3.31 folds, respectively. Temozolomide, AZD3463 and the combination arrested cell cycle G1/S at different ratios. Our results suggested that

AZD3463 combination may be an alternative approach to dose reduction of TMZ for GBM cells.

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P12.068A

Combination of AZD3463 and salinomycin can induce apoptosis and cell cycle arrest on glioblastoma multiform cells

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Introduction: Glioblastoma multiforme (GBM) is the most common malignant and aggressive brain tumor with limited efficient treatment options. AZD3463 is a potent ALK/IGF1R inhibitor designed to overcome the acquired resistance to crizotinib, while Salinomycin, an ionophore antibiotic, has been identified as a novel alternative to traditional anticancer drugs and a potential Wnt/β-catenin pathway inhibitor. In this study, we aimed to investigate the combinational effects of AZD3463 and salinomycin on T98G GBM cell line.

Materials and Methods: The cytotoxicity of AZD3463 and salinomycin on T98G cells were determined with WST-8 assay. The effects of the combination of AZD3463 and salinomycin on apoptosis and cell cycle were determined by AnnexinV-FITC Apoptosis Detection Kit and BD Cycletest Plus DNA Reagent Kit, respectively.

Results: IC_{50} doses of AZD3463 and salinomycin were found as 529 nM and 7.3 μM for 48h, respectively. Combination doses of AZD3463 and salinomycin were found as 3.3 μM and 333 nM with 0.673 combination index, respectively. While IC_{50} doses of AZD3463 and salinomycin induced apoptosis 1.2 and 1.4-fold, as the combination dose induced apoptosis 3.2-fold, compared to control. While IC_{50} dose of salinomycin did not affect, AZD3463 and the combination dose arrested the cell cycle at G1 phase.

Conclusions: We suggest that the combination of AZD3463 and salinomycin via concurrently regulating signal pathways ALK and Wnt, may be a novel alternative approach in the treatment of GBM.

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P12.069B

ETV1 oncogene fused to novel gene partner PTPRZ1 in a case of pilocytic astrocytoma

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Gliomas are highly aggressive brain tumours, exhibiting extreme genetic and clinical heterogeneity. It is these variabilities that represent a great challenge in their diagnosis and treatment, and are major causes of high mortality. Development and advances of high-throughput sequencing techniques identified several new single-nucleotide variants and gene rearrangements. Characterization of gene fusions and how they affect cellular processes in glioma oncogenesis will help us extend our knowledge of tumour biology and contribute to more personalized and targeted treatment of the disease.

Routine NGS analysis of the FFPE tissue sample of pilocytic astrocytoma in a 7-year old boy revealed a fusion between the *ETV1* oncogene and the *PTPRZ1* gene. To the best of our knowledge, there is no mention of the PTPRZ1-ETV1 (PE) fusion in glioma or any other type of cancer to date. In order to validate the PE-fusion, we used PE-fusion-specific primers for RT-PCR and subsequent Sanger sequencing.

Both *ETV1* and *PTPRZ1* genes are thought to act as oncogenes. *ETV1* is a member of ETS family of transcription factors, known tumour-promoting factors of Ewing sarcoma and prostate tumours. *PTPRZ1* is considered as a tumour growth-promoting oncogene in glioma. In 8-15% of glioma, *PTPRZ1* is fused to the *MET* oncogene - a ZM-fusion, which is associated with poorer prognosis but also represents a positive predictive biomarker for treatment with kinase inhibitors. Regarding all, PTPRZ1-ETV1 fusion presents a novel possible target for glioma treatment; however, functional studies are needed to determine the impact of the fusion in pathogenesis of glioma.

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P12.070C

FOCAD is associated with survival in IDH-mutant astrocytic gliomas, localizes to centrosomes, and impacts microtubules

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In search of novel genes associated with glioma tumorigenesis, we have previously shown that *KIAA1797/FOCAD* is frequently deleted in gliomas and that the encoded focadhesin functions as a tumor suppressor impacting proliferation and migration of glioma cells *in vitro* and *in vivo*. Here, we examine an association of *FOCAD* loss with overall survival of glioma patients, and address the molecular mechanisms that govern the suppressive effect of focadhesin on glioma tumorigenesis. We found *FOCAD* loss to be associated with profoundly inferior outcome in patients with isocitrate dehydrogenase (IDH)-mutant WHO grade II to IV astrocytic gliomas. Multivariable analysis of WHO grade II to IV astrocytic gliomas confirmed *FOCAD* loss as an independent prognostic factor for overall survival. Subsequently, using a yeast two-hybrid screen and pull-down assays, we identified tubulin beta-6 and other members of the tubulin family as novel focadhesin-interacting partners. We demonstrate that tubulins and focadhesin co-localize at the centrosome, and that focadhesin is enriched in proximity to centrioles. Focadhesin also localizes to microtubules via its interaction partner SLAIN motif family member 2, and reduces microtubule growth velocity, possibly explaining why focadhesin decreases cell migration. During the cell cycle, focadhesin levels peak prior to mitosis and influence time-dependent mitotic progression, providing a possible explanation for focadhesin-dependent cell growth reduction. We conclude that *FOCAD* loss is associated with decreased overall survival in subsets of glioma patients, a prognostic effect that may be linked to our findings that focadhesin physically interacts with tubulin family members, impacts microtubule assembly and mitotic progression.

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P12.071D
Study of SEZ6L molecular alterations in solid tumors

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Introduction: The SEZ6L gene has been recently described to be altered in lung tumors. We set out to test if this is true for other types of tumors as well.

Materials and Methods: We studied SEZ6L by quantitative Polymerase-Chain Reaction (qPCR) on tumor-derived DNA samples. In total, we studied: 55 female and 18 male breast tumors, 30 grade II-III and 30 grade I endometrial tumors 38 ovarian tumors.

Results: We identified SEZ6L amplifications in 1 (1.7%) of female breast cancers, 3 (16.7%) of male breast cancers, 1 (3.0%) of grade II-III and 2 (6.6%) of grade I endometrial cancers and 3 (7.9%) of ovarian cancers. We found SEZ6L heterozygous deletions in 23 (39.7 %) of female breast cancers, 4 (22.2%) of male breast cancers, 10 (33.3%) of grade II-III and 3 (10.0%) of grade I endometrial cancers and 19 (50.0%) of ovarian cancers We described SEZ6L homozygous deletions in 22 (37.9%) of female breast cancers, 6 (33.33%) of male breast cancers, 5 (16.6%) of

grade II-III and 5 (16.6%) of grade I endometrial cancers and 4 (10.5%) of ovarian cancers.

Conclusions: Though SEZ6L had, until now, only been shown to be altered in lung cancer, we show that a great percentage of gynecological tumors present alterations, thus, potentially linking SEZ6L with these types of tumors.

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P12.073B

The identification of pathogenic variants in BRCA1/2 negative, high risk, hereditary breast and/or ovarian cancer patients

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Introduction: In the majority of hereditary breast and /or ovarian cancer (HBOC) patients the genetic predisposition is unknown. Currently extensive research focusses on the identification of pathogenic variants causative for the development of the disease.

Materials and Methods: NGS-based multiple gene panel resequencing in combination with a high resolution CGH-array was used to identify genetic risk factors for HBOC in 237 high risk patients who were previously tested negative for pathogenic *BRCA1/2* variants.

Results: We identified 32 pathogenic variants in 14 different genes (*ATM, BLM, BRCA1, CDH1, CHEK2, FANCG, FANCM, FH, HRAS, PALB2, PMS2, PTEN, RAD51C* and *NBN*) in 30 patients (12.7%). Two pathogenic *BRCA1* variants that were previously undetected due to less comprehensive and sensitive methods were found. Five pathogenic variants are novel, three of which occur in genes yet unrelated to hereditary breast and/or ovarian cancer (*FANCG, FH* and *HRAS*). In our cohort we discovered a remarkably high frequency of truncating variants in *FANCM* (2.1%), which has recently been suggested as a susceptibility gene for hereditary breast cancer. Two patients of our cohort carried two different pathogenic variants each and 10 other patients in whom a pathogenic variant was confirmed also harbored a variant of unknown significance in a breast and ovarian cancer susceptibility gene.

Conclusions: With our screening strategy, we were able to identify pathogenic variants predisposing for tumor formation in 12.3% of *BRCA1/2*-negative breast and/or ovarian cancer patients.

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P12.074C

Prevalence of genetic susceptibility for breast and ovarian cancer in a non-cancer related study population: secondary germline findings from a Swiss single center cohort

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Objective: For the first time, in a retrospective non-cancer related cohort of a single Swiss genetic center, we systematically assessed the prevalence of secondary findings in 19 susceptibility genes for hereditary breast and ovarian cancer (HBOC).

Design: A total of $n = 400$ non-cancer related individuals undergoing whole-exome sequencing were included after quality assessment (mean age of 43 years). The majority of the cohort were Caucasian (n = 336, 84.0%), for $n = 170$ individuals (42.5%) of which an autochthonous Swiss descent was assumed. Each rare variant (MAF≤0.65%) was manually evaluated according to the ACMG-AMP

standards including "hypomorphic" as additional distinct pathogenicity class.

Results: Overall, 526 rare secondary variants were encountered with the *BRCA1/2* genes accounting for 27.2% of the total variant yield. If stratified for variant pathogenicity, for *BRCA1/2*, three pathogenic variants were found in three females of Italian ancestry (carrier frequency of 0.8%). In the 17 non-*BRCA* genes, five carriers of (likely) pathogenic variants (1.3%) were identified with two Swiss individuals harboring the *CHEK2* Arg160Gly variant being known as recurrent among Caucasians. Hence, the overall carrier rate of a (likely) pathogenic variant summed up to 2.0%. Additionally, seven various hypomorphic alleles were detected in 22 individuals (5.4%).

Conclusion: Herein, we provide first evidence for a high prevalence of HBOC-related cancer susceptibility in the heterogeneous Swiss general population and relevant subpopulations, particularly in individuals of Italian descent. These pioneer data may further substantiate considerations about population-based HBOC screenings. This study was funded by a von Sick CardioOnco grant.

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P12.075D
Multiple genetic tumor syndromes: when to suspect them?

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The use of multigene panels allowed the identification of carriers of pathogenic variants (PVs) in 2 or more cancer predisposing genes (CPGs). The condition has been named Multiple Inherited Neoplasia Alleles Syndrome (MINAS): here we present 6 new cases and discuss the implications. The *NF1* gene was involved in two cases, associated with *RET* and *MSH2* PVs, respectively. Both patients presented clinical NF1 in addition to positive family history (known familial *RET* PV) and personal and family history of early-onset colon cancer, respectively. The latter patient also developed three independent soft tissue sarcomas, that are uncommon in both NF1 and Lynch syndrome. The other cases involved *BRCA1* and/or *BRCA2:* 1) early-onset breast cancer, ovarian cancer (*BRCA1* + *BRCA2*); 2) macrocephaly, skin lesions, colon cancer, pancreatic cancer in the proband, whose father had breast cancer (*BRCA2* + *PTEN*); 3) early-onset breast cancer and renal cancer with lung cysts in 2 siblings (*BRCA1* + *FLCN*); 4) bilateral breast cancer, pheochromocytoma, and medullary thyroid cancer (*BRCA1* + *RET*). Overall, in 5 patients we actively searched for a second PV whereas the diagnosis was incidental in one case. Our data indicate that the phenotypes of patients with Multiple Genetic Tumor Syndromes (MGTS) can be the sum of the different syndromes or driven by one of the two genes or they might represent a multiplicative effect. MGTS should be actively searched in all patients with complex/atypical phenotypes or a suggestive family history, that cannot solely be explained by defects in a single CPG.

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P12.076A
Exome sequencing in familial cancer patients; yield, advantages and disadvantages of implementation in the clinical practice

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Introduction: For many patients from families with strong suspicion for hereditary cancer no pathogenic variant can be found using single gene or small gene panel sequencing methods.

The aim of this study is to evaluate the yield of exome sequencing in patients in whom standard diagnostics did not lead to a molecular diagnosis. Furthermore, we discuss advantages and disadvantages of implementation of exome sequencing in clinical practice.

Materials and Methods: Exome sequencing was performed in 130 patients with a strong suspicion for hereditary cancer who had tested negative in previous genetic tests for germline variants. A panel of 209 genes proven to be causal for cancer or associated with cancer was analyzed.

Results: We identified 10 pathogenic variants in 130 patients (7.7%) which are (partially) causative of the phenotypes in the families. These ten pathogenic variants were found in *ATM* (3 times), *LZTR1*, *PALB2*, *PMS2* (2 times), *MUTYH*, *SUFU*, *TGFBR1*. Additionally, 22 pathogenic/deleterious variants were identified which did not match the family's cancer phenotype. Furthermore, we selected some

variants with high prior probability of pathogenicity based on the function of the gene, in silico data and frequency of the variant in control cohorts for further research.

Conclusions: Our study shows that exome sequencing, also after standard tests, is valuable in identifying the causative pathogenic variants. However, given the associated costs and workload for clinical molecular geneticists at this moment, it should only be offered to specific families when targeted analysis of the most relevant genes is negative.

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P12.077B

Germline mutations in *POLE* and *POLD1* associated with hereditary cancer

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Introduction: Germline mutations in the exonuclease domains of polymerases *POLE* and *POLD1*, which affect their proofreading capabilities, predispose to adenomatous polyps, colorectal cancer, endometrial tumors and other extracolonic malignancies. Tumors and polyps with exonuclease domain mutations exhibit an increased mutation rate (hypermutation) and a specific associated mutational signature. We aim at defining the tumor spectrum of the syndrome, assess the prevalence of *POLE/D1* pathogenic mutations in hereditary cancer, and evaluate the challenge of variant classification for these genes.

Materials and Methods: We studied a total of 1,525 unrelated probands referred for genetic counseling: a prospective cohort of 1,021 hereditary cancer patients subjected to a multi-gene hereditary cancer panel, which included the whole sequence of *POLE* and *POLD1*; and 504 patients without mutations in known cancer-predisposing genes, selected according to their phenotypic characteristics, where *POLE/D1* exonuclease domains were directly sequenced. Co-segregation analyses, case-control allele frequencies, yeast-based assays and tumor mutational analyses (mutation burden and mutational signatures) were performed for variant interpretation.

Results: We identified 8 missense variants within the exonuclease domain, 4 loss-of-function and 5 missense predicted deleterious variants outside the exonuclease domain; all of them with population allelic frequencies <1%. Pending interpretation of the clinical impact of loss-of-function and non-exonuclease domain mutations, our results, implemented in the ACMG/AMP guidelines for variant classification, allowed us classify 2 variants as pathogenic, 2 as benign, and 4 as variants of unknown significance.

Conclusions: Our study reveals relevant challenges for the classification of *POLE/POLD1* variants, which need to be considered for routine genetic diagnostics.

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P12.079D

HTRA4 could be involved in early-onset breast and ovarian cancer

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Introduction: Early-onset breast cancer shows clinical and molecular differences when compared with other types of breast cancer and, as such, could be considered a breast cancer subtype. Our aim was to find gene regions that were altered in early-onset breast cancer and, within those regions, study candidate genes.

Materials and Methods: Early-onset breast cancer samples were studied by comparative genomic hybridization (CGH) arrays and candidate gene was studied by quantitative polymerase chain reaction (qPCR) in 22 early-onset breast and 15 ovarian cancer samples. Also, we analysed the same candidate gene by qPCR in nine breast cancer cell lines: HCC1569, MDAMB415, MDAMB231, Bt474, Bt549, MCF7, AU565, HBL100 and SUM149.

Results: The CGH arrays showed deletion of 8p11.22 in 22% of samples. However, HTRA4 (a gene located in this locus) qPCR studies showed abnormal amplification in 81.8% of breast cancer samples and in 80% of the ovarian cancer ones. Moreover, this gene was amplified in three of the nine cell lines studied and deleted in other five cases.

Conclusions: Our results show for the first time that HTRA4, a gene involved in trophoblast implantation and deregulated in eclampsia, could also be involved in early-onset breast and ovarian cancer.

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P12.080A

Studying the Immunological synapse in GBM - The expression of checkpoint genes and transcription factors involved in immunological synapse formation in GBM in adults

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The complex immunological synapse between T lymphocytes and cancer cells contains checkpoint proteins that modulate the signal transmitted to T lymphocytes. Not all patients respond to the checkpoint inhibitors treatment, especially in glioblastoma multiformae (GBM). The expression of checkpoint genes is variable and prone to the regulation of yet unrevealed mechanisms. Our study is aimed at evaluating the transcriptional and post-transcriptional regulation of checkpoint mRNAs. We analysed the expression of mRNAs from 617 adult GBM samples from the TCGA, as well as from 4 cell lines by RT-PCR. Of 22 mRNAs suggested in the literature to be expressed on the 'cancer side' of the immunological synapse, only a small fraction seem to be expressed in GBM, using a pre-defined expression cutoff, some of which were not mentioned in GBM literature so far. The expression of several genes is upregulated, eg.PD-L2, compared with normal tissue data, and seem to be co-expressed together. Other genes are significantly down-regulated or not expressed at all eg.CD80, suggesting the possible reason for many failures of checkpoint inhibitors immunotherapy in GBM. Our results may suggest that most of the genes involved in the formation of immunological synapse are significantly down-regulated or not expressed at all in GBM, providing the potential explanation for many failures of checkpoint inhibitors immunotherapy in GBM. Studying the crosstalk between checkpoint genes and their regulation might be crucial for improving immunotherapy. Revealing novel interconnections may aid in the development of new diagnostic tools, outcome predictors, immunotherapeutic drugs or combinations.

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P12.081B

Incidental findings in oncogenetics: results and impact on patients

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Introduction: With next generation sequencing, physicians are confronted to more complex and uncertain data, including incidental findings (IF). Several recommendations about the return of IF have been published. Some professional societies encouraged the use of panels to avoid IF detection. Little is known about the impact of such results on the patients in the context of diagnostic panel testing in oncogenetics.

Material and Methods: From June 2014 to July 2018, 2500 patients with an indication of search for genetic predisposition to cancer benefited from a 25 to 47 cancer genes panel. A semi-structured questionnaire was proposed to patients in which an IF was detected at distance of the results to assess the understanding of the result, the change in medical care, the communication of IF to the family, the psychological impact at time of diagnosis and at distance. This interview was conducted by phone by a physician/genetic counsellor.

Results: Twelve adult patients had the return of an IF in a cancer predisposition gene (*RAD51C, PMS2, SDHC, RET, BRCA2, CHEK2, CDKN2A, CDH1, SUFU*). To date, phone interviews have been conducted for 7 patients. Most of them had been surprised by the result, but not anxious. They all began the recommended follow-up and did not regret the procedure. Transmission of the information to their offspring was systematic but more difficult to siblings or second-degree relatives.

Conclusion: IF will be inherent to the development of new technologies. It is important to increase our knowledge on the impact of such results in different contexts.

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P12.082C
JAK2 V617F mutation, cytogenetic abnormalities and endogenous erythroid colony formation in patients with polycythaemia vera

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Introduction: Polycitemia vera (PV) is a chronic myeloproliferative neoplasm characterized by increased red blood cellc. The most frequent genetic abnormality is the somatic mutation of Janus kinase 2 gene (JAK2 V617F) and it occurs in more than 90% of patients with PV.The aim of this study was to investigate the frequency of JAK2 V617F gene mutation in patients with PV and to compare results with presence of cytogenetic abnormalities and endogenous erythroid colony (EEC) formation.

Materials and Methods: Peripheral blood and bone marrow samples of 65 patients with PV were analyzed. The diagnosis of PV was established according to the bone marrow criteria of the World Health Organization. Mutation of JAK2 V617F was determined by allele specific pcr analysis. For detection of EEC formation we used assays of human clonogenic heamatopoietic progenitor cells with agar-leukocyte conditioned medium without recombinant human erythropoietin. Cytogenetic analysis was done according to standard procedures.

Results: Mutation of JAK2 V617F was found in the samples of the peripheral blood in 61/65 (93.8%) PV patients. Cytogenetic abnormalities, trisomy 9 and del (20q), was detected in 2/65 (3 %) patients. EEC formation was obtained in the sample of bone marrow in 59/65 (9O,8%) PV patients. In 57/65 (87,7%) patients we detected presence of EEC formation and mutation of JAK2 V617F at the same time.

Conclusions: Presence of JAK2 V617F mutation and EEC are essential characteristics of PV. Considering these results, we concluded that the EEC formation observed in PV could be partially due to the JAK2-dependent activation signaling pathway.

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P12.083D
Laryngeal metastasis and relapse development predicted by a (epi)genomic signature

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Introduction: The survival of laryngeal cancer patients and the target therapies available remain poor. So, the identification of early diagnostic and prognostic biomarkers and the development of molecular models to distinguish patients that will recur and/or develop metastasis after treatment as well as to benefit with target therapies is of utmost importance to decrease mortality, improve survival rates and quality of life. This study aimed to develop a model of recurrence and metastasis prediction using a genomic and epigenetic characterization of laryngeal cancer samples.

Material and Methods: The genomic and epigenetic characterization of 21 tumor samples from laryngeal cancer patients was performed using array comparative genomic hybridization technique, Agilent 4x180K and methylation-specific multiplex ligation dependent probe amplification technique. The most common amplification and deletion chromosomal regions and the respective altered genes were identified. A genomic and epigenetic signature that distinguishes between patients with metastasis/recurrence from those without, was determined using a logistic regression classifier with balanced training and test sets.

Results: Several chromosomal regions and genes were observed with copy number alterations and methylation. The developed predictive genomic and epigenetic model comprises the 3p chromosomal region and *WT1*, *VHL* and *THBS1* genes, highlighting a molecular signature with clinical applicability.

Conclusion: This model for recurrence and metastasis development may help in a more practical and individualized laryngeal cancer patient management, contributing to a more targeted drug design and, ultimately, improving patients' quality of life.

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P12.084A
Investigation the expression of hypoxia inducible factors and embryonic stem cell genes in patients with laryngeal carcinoma

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Introduction: Laryngeal cancer accounts for about 25% of head and neck cancers and is usually seen in men of middle or older age. More than 90% of laryngeal cancers are squamous-cell cancers. One of the most common features of solid tumors and one of the major environmental stresses is hypoxia. The reduction of the oxygen concentration below the physiological level, promotes maintenance and self-renewal of the human embryonic stem cells, and reduces spontaneous differentiation in vitro. Similarities between stem cell and cancer cells suggest that both groups may use common pathways in the regulation of gene expression. In this study, we aimed to determine the expression levels of hypoxia-induced factors HIF-1α and HIF-2α and embryonic stem cell genes SOX2, OCT4, NANOG and ESRRA in laryngeal cancer patients.

Material and Methods: Twenty six patients with squamous cell laryngeal carcinoma were included in this study. Real Time PCR was performed to compare the expression levels of hypoxia inducible factors and embryonic stem cell genes in tumor tissues and adjacent normal tissues.

Results: It was determined that the expression of HIF-1α, OCT4 and NANOG genes were increased significantly in tumor tissues compared to adjacent normal tissues. HIF-2α, SOX2 and ESRRA gene expressions did not show significant differences between tumor and adjacent normal tissues.

Conclusion: Our results show that there may be a relationship between the clinopathologic features of the disease and hypoxia inducible factors and embryonic stem cell genes in the pathophysiology of the laryngeal cancer.

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P12.087D
Comprehensive Detection of Germline and Somatic Structural Mutation in Cancer Genomes by Bionano Genomics Optical Mapping

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Introduction: The ability to identify structural variants (SVs) is crucial in cancer genetics. Karyotype and cytogenetics are manually intensive. Microarrays and sequencing cannot detect calls in segmental duplications

and repeats, and miss balanced variants and low-frequency mutations.

Materials and Methods: We describe the Bionano Genomics's Saphyr platform to identify SVs in cancer genomes. DNA >100 kbp is extracted, labelled at specific motifs, and linearized through NanoChannel arrays. Molecule images are digitized and de novo assembled, creating chromosomal-arm scale genome maps. Cancer mutations >500 bp are detected by aligning the molecules or the genome maps to the public reference.

Results: Over the past 12 months, the power of Bionano's cancer workflow has been demonstrated on nearly 50 various cancers, including leukemia, breast, ovarian, prostate, pancreatic, among others. While the number of SVs varies among samples, we typically observe >5500 calls per genome. Among leukemia samples, we captured the BCR-ABL1 translocation as well as deletions impacting tumor suppressor genes such as PTPN14 and ESRRG. We resolved the structure of large duplications (790 kbp) disrupting BRCA1 in early-onset breast cancers, found the amplification of MYC in lung cancers.

Conclusions: In conclusion, with one platform, Saphyr can discover a broad range of traditionally refractory but relevant SVs, and improves our understanding of cancer.

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P12.088A
Development of Cancer Surveillance Guidelines for *TP53* carriers: Recommendations from the UK Cancer Genetics Group Consensus Meeting

H. Hanson, UK Cancer Genetics Group, LFS Surveillance Consensus Day attendees

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Li-Fraumeni syndrome (LFS) is a rare inherited condition caused by pathogenic variants in *TP53*. Individuals with LFS have significantly increased risks of cancer; 41% by age 18 and approaching 100% by 70.

To date, in the UK the only national surveillance recommendation for LFS is for breast MRI. However, there is increasing evidence for more comprehensive surveillance, including whole body MRI (WB-MRI) with increased cancer detection and survival.

International recommendations were published by Kratz *et al*. (2017). However, these have not yet translated into NHS practice.

The UK CGG therefore convened a National Consensus Group meeting on 6th July 2018 to agree a consistent national approach to surveillance.

A pre-meeting survey was sent to 24 Regional Genetics centres (UK and Ireland). The survey results and the recommendations made by the International Group were discussed in depth at the meeting by 43 Clinical Geneticists, Genetic Counsellors, Oncologists, Radiologists and Patient representatives.

Prior to the Consensus meeting, less than 50% of centres agreed with the International recommendations, with only 25% of centres agreeing with annual WB-MRI. However, the group recognised that due to the rarity of LFS, there is a limited evidence base to support screening in terms of early detection and cancer mortality. On the day, consensus was reached to follow the international recommendations, with the exception of gastrointestinal surveillance. A surveillance protocol for children and adults with LFS and best practice guidelines for WB-MRI in the UK were established.

The survey results, recommendations and the clinical implementation will be presented.

H. Hanson: None.

P12.089B
Genetic modeling of Li-Fraumeni syndrome in Xenopus tropicalis

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Introduction: CRISPR/Cas9 mediated genome editing generates unique opportunities in human disease modeling. It is now possible to create functional gene knockouts in a number of model organisms. *Xenopus tropicalis* is an aquatic organism favorable for modeling human disease due to its diploid genome,, high progeny numbers and fast embryonic external development. Li-Fraumeni Syndrome (LFS) is a disorder, that predisposes for several malignancies like sarcomas and lymphomas. Our aim is to examine whether a *tp53* mutant *X.tropicalis* line could mimic LFS linked afflictions.

Materials and Methods: A *tp53* mutant *X.tropicalis* line was generated by injecting *tp53* sgRNA precomplexed with Cas9 protein. Injected embryos were raised until sexual maturity and *tp53* heterozygous and homozygous mutant progenies were raised. Animals were closely monitored for external signs of disease. Animals that were lethargic or exhibited decreased body weight or enlarged abdomen were euthanized and histologically processed.

Results: Homozygous *tp53* mutant animals showed rapid morbidity compared to their heterozygous counterparts. In 75% (n=8) of $tp53^{-/-}$ animals, disturbed architecture of the spleen was determined by CD3 and PCNA immunohistological analysis, implying the presence of hematologic malignancies. Furthermore, flow cytometric analysis of blood in one out of six non-moribund mutants showed enrichment of CD3/CD8-positive cells, indicative of T-cell leukemia. Besides hematological malignancies, animals demonstrated liposarcoma and high grade undifferentiated spindle and round cell sarcoma.

Conclusions: Our *tp53* mutant line recapitulates several LFS-p53 related malignancies. Our model could offer a platform for identification of modifier genes and provide a sensitized background for screening for novel cancer drivers.

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P12.090C
Whole-body MRI for cancer screening in patients with germline *TP53* pathogenic variants - a presentation of the Swedish surveillance program including two case reports

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Current guidelines in Sweden regarding individuals with a pathogenic or likely pathogenic germline *TP53* variant recommend patients to take part of the national Swedish P53 Study (SWEP53). All known adult eligible carriers, regardless of age, are offered to take part in a surveillance program, offering yearly whole-body, breast, brain MRIs and breast ultrasound. A special surveillance program for individuals <18 years old with a 50% risk of being a mutation carrier or with a verified *TP53* variation, includes ultrasound of the abdomen and urine corticosteroid profiles. Further clinically motivated examinations are performed when needed. In a submitted publication, we present the surveillance program within the SWEP53 including further work-up by describing two case reports - one patient with prior malignancies and a healthy carrier with a common benign finding. So far, 25 adults and 9 children have been included in SWEP53 and 24 adults have performed their baseline MRI. In seven of the 24 adults (29%), imaging findings needing further work-up were identified on whole-body MRI at baseline. In Europe, there are surveillance programs within studies such as SIGNIFY (UK) and LIFSCREEN (France), but the SWEP53 is the first structured surveillance program including radiological and clinical routines for *TP53* mutation carriers in the Scandinavian setting.

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P12.091D
Long non coding RNA UCA1 as noninvasive tumour marker for bladder cancer

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Introduction: Bladder cancer (BC) is one of the most common cancers of the urinary tract. Despite improvements in clinical treatment more than 50% of patients are relapsed within the next five years. Therefore we are still looking for the most sensitive and specific marker that would enable quick and precise diagnosis and monitoring of the disease. Long noncoding RNA *UCA1* (lncRNA) plays a special role in the regulation of tumor cell proliferation, differentiation and apoptosis.

Methods: The presence of the *UCA1* was confirmed by the real-time qPCR (quantitative polymerase chain reaction) method in 172 samples (tissue and serum) from patients diagnosed with BC. Analysis of relative gene expression levels was performed using the formula 2^{-dCt} with dCt=Ct (target gene)-Ct (control). The *tata box binding protein* (*TBP*) was used as a hausekeeping gene.

Results: Statistical analyzes were performed using U Mann-Whitney test and ANOVA Kruskal-Wallis test to determine the correlation between clinical and histopathological parameters and primary or recurrent tumor. We found that the lncRNA *UCA1* was significantly higher in both tissue and serum samples of BC patients than in

control group and in primary BC than in recurrent tumor. The increased expression was also associated with high grade and progression. In conclusion, our results indicated that lncRNA*UCA1* could be useful diagnostic and prognostic marker for BC, however further studies with larger cohorts are necessary to evaluate it. One of the limitations of our research is low number of tissue samples from control group.

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P12.092A

Genome-wide survival analysis of surgically-treated lung adenocarcinoma patients

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Background: Lung cancer patients with the same pathological stage and same treatments often show different survival patterns for unknown reasons. We hypothesized that these differences are due to individual germline polymorphisms modulating still unknown genetic mechanisms affecting cancer growth and metastasis.

Methods: To test the role of genetic constitution in individual risk of poor prognosis, we carried out an overall survival genome-wide association study in 584 surgically-treated lung adenocarcinoma patients, genotyping >2-million single nucleotide polymorphisms (SNPs). For 232 samples, we had also available transcriptome data from the non-involved lung tissue, that we used to test expression quantitative locus (eQTL) activity of SNPs associated with survival.

Results: From the analysis of 1,415,218 SNPs passing quality control steps, we found four SNPs (rs75767414, rs11074720, rs147882561, and rs79851088) associated with overall survival at nominal $P < 1.0 \times 10^{-6}$. A multivariable Cox's model including all the four associated SNPs, together with clinical covariates, including pathological stage, showed that each of the four SNPs acts as an independent risk factor for overall survival. None of the candidate SNPs acted as a cis-eQTL, whereas three SNPs modulated expression of target genes in trans ($P < 1.0 \times 10^{-4}$).

Conclusion: Overall survival of surgically-treated lung cancer patients is modulated by individual genetic constitution. We are now applying random forest models to investigate complex interactions between SNPs that could explain the observed variability in patient survival. Understanding the mechanisms underlying individual risk of poor prognosis may help discovering new tools to improve survival of lung cancer patients.

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P12.094C

Preliminary results from a prospective study of germline alterations in early onset lung cancer patients (EOLUNG study)

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Introduction: Non-small lung cancer (NSCLC) is a rare disease among young adults. Germline DNA mutations have not been systematically studied in this population. Next generation sequencing (NGS) could be useful to determine the proportion of young patients with NSCLC harboring germline mutations.

Patients and Methods: This multicentre prospective study aims to assess whether NSCLC patients aged <51 years at diagnosis harbor germline mutations using a custom NGS panel (I2HCP) developed in our center. This panel covers 136 genes related with hereditary cancer. The enrolment begun on June 2018 and a total of 43 patients have been recruited.

Results: Here, we present an initial analysis based on the first 26 recruited patients. First, a restricted analysis focusing on 26 genes involved in germline NSCLC yielded a

total of 40 variants of uncertain significance (VUS) and 2 putative pathogenic mutations (PPat) in *BLM* and *NBN*. Next, an analysis of the whole 136-gene panel was performed and 5 additional PPat mutations were detected in *FANCA, MUTYH, NTHL1, RIT1* and *SBDS*. In total, 7 putative PPat mutations were identified in 6 patients (23% of patients from this initial cohort of 26 NSCLC patients). All variants are still being evaluated at clinical level. The final results will be presented at the Congress.

Conclusions: Based on these preliminary results, the I2HCP gene panel is useful to identify young patients with NSCLC harboring cancer hereditary predisposition. Further validation in a larger cohort of NSCLC young adults is warranted.

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P12.095D

Induced genotoxicity produced by flavonoids and novel Cu(II)-flavonoid complexes in human lymphocytes

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Flavonoids (FL) are potent antioxidants, free radical scavengers, metal chelators, and lipid peroxidation inhibitors exhibiting several beneficial biological activities [1]. The flavonoid chrysin is a flavone found in honey and propolis, and actively investigated for its potential biological effects against Parkinson's disease and the proliferation of cancer cells, as well as on the regulation of multiple cell signaling pathways [2]. Research on the pivotal role of stable Cu(II)-complexes (CuCo) with physiological substrates such as FL, in cell physiology as catalytic cofactors in the redox chemistry of mitochondrial respiration, iron absorption, free radical scavenging, and elastin cross-linking have provided new insights in the field of copper homeostasis and in particular into the understanding of intracellular trafficking and distribution of copper at molecular levels [3]. In this work two novel hybrid Cu (II)-chrysin complexes were synthesized, isolated and physico-chemically characterized through single crystal X-ray diffraction, FT-IR, UV-Vis, fluorescence, TGA, and ESI-MS measurements. Genotoxicity estimated by the frequency of Sister Chromatid Exchanges (SCEs), cytostaticity based on the Proliferating Rate Indices (PRIs) and cytotoxicity based on the Mitotic Indices (MIs) at human peripheral lymphocyte cultures, were all significantly higher both in the case of FL and CuCo. Irinotecan (CPT) was also utilized as standard genotoxic agent and the combination of FL or CuCo plus CPT provided a mechanism by which the rate of genetic damage can be induced. The novel hybrid materials exhibit enhanced solubility and bioavailability, and increased anti-cancer activity compared with free chrysin and probably gives rise to fruitful bioapplications and perspectives.

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P12.096A

Pitfalls in Lynch syndrome genetic testing: a case of a polymorphic variant in primer-annealing sequence leading to the wildtype allele dropout in *MLH1* gene

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PCR-based screening techniques, like Sanger sequencing and NGS are considered gold standard methods for detection of pathogenic variants in hereditary cancer syndromes. Polymorphic variants represent a risk for

misdiagnosis in genetic testing, since the presence of these variants in primer-annealing sites may cause allele-dropout. We present a Lynch syndrome family fulfilling Amsterdam II criteria. The proband was diagnosed of endometrial cancer at age 39. Genetic diagnosis of Lynch syndrome was performed by Sanger sequencing. A pathogenic variant was detected in exon-19 of *MLH1:* c.2150_2153dupAACA, with a clear homozygous pattern. Predictive genetic testing was performed in eight relatives. Unexpected results were found. Proband´s mother presented the same homozygous pattern for the pathogenic variant, while the proband´s father was homozygous wildtype. Furthermore, proband´s brother had also a homozygous pattern for the pathogenic variant, while his son was homozygous wildtype. We considered the possibility of allele-dropout of the wildtype allele due to a polymorphism in the primer-annealing sites. Proband was reanalyzed by NGS with hybridization/capture target enrichment. We evidenced a heterozygous indel polymorphism in the annealing sequence of the reverse primer: c.*35_*37delCTT. Adequate external primers for *MLH1*-exon-19 were designed. The proband and her relatives were re-tested by Sanger sequencing. We confirmed that pathogenic and indel variants are in *trans* phase. Consequently, the patients previously considered homozygous for the pathogenic variant were indeed heterozygous. We would like to remark the importance of be aware of, and try to minimize, the limitations of genetic testing and consider to investigate deeper when unexpected findings are detected.

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P12.097B
An unusual presentation of Lynch Syndrome in teenagers

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Introduction: Heterozygous mutations in the mismatch repair (MMR) genes cause Lynch syndrome. Phenotype and age of at cancer diagnosis differs among the various genes, however mean age of cancer onset is 44-61 years and surveillance is recommended from age 20-25 or 2-5 years prior to the earliest family reported malignancy (NCCN guidelines V1.2018). Bi-allelic MMR mutations cause CMMRD characterized by childhood malignancies and NF1 features. We present two patients, both with early age onset malignancies, diagnosed with Lynch syndrome but with no evidence of CMMRD.

Patient 1was diagnosed at age 15 with poorly differentiated endometrial endometriod carcinoma of mullerian origin. A clonal loss of MSH6 was found in the tumor. This result was suggestive of a biallelic somatic mutation, typically present with a driver POLE mutation. Multi-cancer NGS testing revealed a heterozygous c.2906A>G mutation in *MSH6*. No *POLE* germline mutation was found. The mutation was inherited from the patient's mother.

Patient 2 was diagnosed with aggressive T cell lymphoma at age 16. NGS cancer panel testing revealed a heterozygous mutation c.1865C>T in *MSH2*, inherited from his father. He had no other pathogenic variants.

Discussion: Malignancies associated with Lynch syndrome usually develop after age 25. *MSH6* associated Lynch syndrome manifests even later. Both patients were diagnosed with cancer before age 20 and had no evidence of CMMRD. Cancer diagnosis in the second decade is rare among Lynch carriers. Such cases should be reported and further explored.

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P12.098C
Reduced *PMS2* expression decreases significantly mismatch repair efficiency

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Introduction: Inherited mutations affecting DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* and *PMS2* cause Lynch syndrome (LS). Yet, the cancer susceptibility associated with *PMS2* mutations differ from the typical LS. The relatively small number of LS families found to segregate *PMS2* mutations and the associated low disease penetrance suggest that *PMS2* may not be an important susceptibility gene. The effect of decreased *PMS2* mRNA expression on MMR capability was determined by *in vitro* MMR assay.

Materials and Methods: Human fibroblasts were transfected with four different *PMS2*-specific shRNA targets to create knockdown (KD) cell lines with varying levels of *PMS2* mRNA expression. A shRNA vector, with no known target specificity in the human genome, was used as a control. Quantitative PCR was carried out using Taqman® assays for *PMS2* with *GAPDH*, *HPRT1* and *ACTB* as

reference genes. The repair efficiencies of the selected KD protein extracts were measured by the *in vitro* MMR assay.

Results: KD cell lines retaining 19%, 33% and 53% of *PMS2* expression demonstrated significantly reduced MMR efficiency compared to their control cells. Surprisingly, cells retaining 53% of *PMS2* expression indicated the most severe problem in the repair capability.

Conclusions: Significant decrease in MMR efficiency due to reduced *PMS2* mRNA expression confirms that *PMS2* is an equally important susceptibility gene among *MLH1*, *MSH2* and *MSH6* genes and that the reduction is efficiently detectable by functional *in vitro* MMR assay.

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P12.099D

Age- and sex-specific cumulative risks of colorectal cancer in Han Chinese patients with Lynch syndrome

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Introduction: Patients with Lynch syndrome have a high risk of colorectal cancer (CRC). In this study, we estimated the penetrance of CRC in Chinese patients with Lynch syndrome.

Materials and Methods: One hundred and thirty-three families comprising 1009 members were collected. Each family was ascertained through a proband with a diagnosed CRC. Of these subjects, 300 were carriers of germline mutations in *MLH1* or *MSH2*, whereas 709 were non-mutation carriers of these mutations. Penetrance of CRC were calculated using modified segregation analysis implemented by Mendel.

Results: The median age at CRC diagnosis were younger in patients with Lynch syndrome than in non-mutation carriers (44.3 vs. 50.4 years, $P = 0.0001$). The cumulative risk (penetrance) of CRC at the age of 70 years were 36.5% (95% CI = 27.7%–46.9%), 34.8% (95% CI = 26.1%–45.5%), and 42.7% (95% CI = 30.1%–57.8%) in the male carriers of *MLH1* or *MSH2*, *MLH1*, and *MSH2* germline mutations, respectively. The penetrance of CRC in the female carriers of *MLH1* or *MSH2*, *MLH1*, and *MSH2* germline mutations were 25.8% (95% CI = 18.6%–35.2%), 24.5% (95% CI = 17.4%–33.6%) and 32.2% (95% CI = 26.3%–39.1%), respectively.

Conclusions: Penetrance of CRC in Chinese patients with Lynch syndrome is 34.8%–42.7% in men and 24.5%–32.2% in women.

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P12.100A

Variant analyses of*PMS2*by single-molecule long-read sequencing

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Introduction: Germline mutations in the mismatch repair gene *PMS2* are a cause of Lynch syndrome. The detection of *PMS2* mutations is challenged by the presence of numerous pseudogenes and frequent gene conversion events. Long-range PCR (LR-PCR) in combination with single-molecule long-read sequencing may overcome the use of multiple DNA- and RNA-based assays for the detection of *PMS2* mutations.

Materials and Methods: We amplified *PMS2* using three LR-PCR amplicons, covering the complete gene with overlapping segments. In order to allow circular consensus sequencing, a method that increases the accuracy of long-reads from 85% to >99%, we limited the size of amplicons to 16kb. Subsequently, twenty cases that showed loss of

PMS2 expression by immunohistochemical staining of their tumor tissue were characterized for the presence of pathogenic variants.

Results: Long-read sequencing is able to detect single nucleotide variants (SNVs), small insertions/deletions, exon deletions >1kb in size and accurate breakpoint mapping. In the rare case a deletion is missed, potentially due to allelic drop-out of deletions spanning the primer binding sites, absence of heterozygosity for SNVs in one of the three LR-PCR amplicons can be used to preselect cases for deletion screening by multiplex-ligation probe amplification (MLPA).

Conclusions: Long-range sequencing is an attractive alternative strategy to accurately detect pathogenic variants and exon deletions in *PMS2*, limiting the use of multiple assays in diagnostics. Furthermore, long-range sequencing has the potential to identify novel pathogenic variants, like intragenic inversions, in cases suspected of *PMS2*-related Lynch syndrome, which thus far have remained without a molecular diagnosis.

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P12.101B
New multidisciplinary tasks in finding all Lynch Syndrome among patients with colorectal cancer below age 70

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Introduction: Lynch syndrome (LS) is the most common cause of hereditary colorectal cancer (CRC). Only ~30% of CRC patients with an increased risk for LS based on age and family history is referred for genetic testing. This may be improved by universal mismatch repair deficiency (dMMR) testing in newly diagnosed CRC below age 70 (CRC<70) followed by germline and somatic testing of MMR genes in those CRC patients with dMMR without *MLH1* hypermethylation (U-MMR workflow).

Methods: The new U-MMR workflow was actively implemented in daily practice of pathologists, surgeons and gastroenterologists in 19 hospitals and 13 pathology laboratories. The feasibility (uptake dMMR-testing, referral rates for genetic testing, testing outcome) and appreciation of patients and clinicians were evaluated.

Results: Pathologists implemented dMMR-testing in 84% (3,025 of 3,602) of CRC<70. Gastroenterologists and surgeons referred 69% of those with dMMR without hypermethylation for genetic testing. Participating clinicians and patients were overwhelmingly positive about the workflow. Among CRC patients with dMMR and without hypermethylation 55% had LS, 43% somatic bi-allelic pathogenic MMR variants (non-hereditary) and for 1% the result was ambiguous. The prevalence of LS was 18% in CRC<40 and 1% in CRC40-70.

Conclusions: Pathologist very well adopted the U-MMR workflow and gastroenterologists and surgeons increased the referral rate of CRC<70 at high risk for LS, although especially in this step the workflow needs improvement. U-MMR workflow in all new CRC<70 is feasible and highly appreciated by patients and clinicians. *The project was supported by the Dutch Digestive Foundation with funding from the 'Vriendenloterij'.*

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P12.103D
Metastatic colorectal cancer chemotherapeutic drugs alter the expression of TGFβ signaling-related miR-17-5p, miR-21-5p and miR-93-5p *in vitro*

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Introduction: The backbone of metastatic colorectal cancer (mCRC) treatment consists of 5-fluorouracil combined with oxaliplatin (FOLFOX) or irinotecan (FOLFIRI). Approximately half of the mCRC patients respond to therapy, so a major challenge remains to identify predictive biomarkers for treatment response. Recent studies revealed an interplay between TGFβ signaling and miRNAs and their role in chemoresistance. The aim of this study was to identify TGFβ signaling-related miRNAs and test their potential as predictive biomarkers.

Materials and Methods: By *in silico* analysis we identified miR-17-5p, miR-21-5p and miR-93-5p which regulate most TGFβ signaling members and show altered expression in CRC. To investigate the effects of anticancer drugs on selected miRNAs expression, SW620 cells were treated

with 5-fluorouracil, oxaliplatin, irinotecan or their combinations for 72h.

Results: MiR-21-5p expression was upregulated while the levels of miR-17-5p and miR-93-5p were downregulated in all treatments. To determine whether this modulatory effect is transient or stable, the miRNA expression was measured on the 1st, 3rd and 6th day after the treatment with drugs combinations. Expression of miR-17-5p and miR-93-5p had a decreasing trend from 1st to 6th day after the treatments, while miR-21-5p expression was upregulated on the 3rd day but was restored close to initial level on the 6th day.

Conclusions: Although tested chemotherapeutic drugs alter expression of all tested miRNAs, only miR-17-5p and miR-93-5p represent potential predictive biomarkers candidates due to their lasting downregulation.

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P12.104A
Investigation of the synergistic effect of metformin and FX11 agents on cell proliferation in PANC-1 cell line

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Introduction: Metformin, an insulin-lowering agent, has been associated with decreased cancer risk in epidemiologic studies in diabetic patients. Interest of metformin in cancer treatment and prevention reflects the recent convergence of several areas of research. Preclinical studies have shown that metformin can inhibit the cancer cells proliferation. Cancer cells take up glucose and after than transform it to lactate even under aerobic conditions, called as the Warburg effect. Sufficient amounts of products of glucose metabolism are required for cancer cell division and rapid growth. Studies showed that, some glycolytic cell types have shown susceptibility to FX11-based lactate dehydrogenase-A inhibition. So, we investigated that effect of metformin and FX11 combination treatment on pancreatic cancer.

Materials and Methods: In this study, sole exposure to metformin or FX11, and their combinations in different are investigated on PANC-1 cell line for determine the effect on cancer growth and progression. MTS assay was performed at 24, 48, 72, and 96 hours to measure time-dependent change of metformin and FX11.

Results: Increased concentrations of metformin and FX11 alone reduced cellular metabolic activity of PANC-1. Interestingly, the combination of metformin and FX11 created a synergistic effect and significantly reduced cell proliferation in vitro.

Conclusions: We found that combination of metformin and FX11 which effect on glucose metabolism reduced the metabolic activity and cancer cell growth. This combination treatment could be change both energetic and apoptotic pathways. Furthermore, five candidate genes for qPCR were identified and three candidate proteins were selected for westernblot.

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P12.105B
Causal variants that underlie miReQTLs in prostate cancer

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The microRNAs (miRNAs) are short non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms. Alterations in the expression of miRNA genes contribute to the pathogenesis of human malignancies incuding prostate cancer. In particular, the expression of miRNAs themselves could be affected by germline variants known as expression quantitative trait loci (eQTLs). These eQTLs exert the allele specific effects on miRNA gene expression (miR-eQTLs), thereby participating in a regulatory role in cancer aetiology. To identify miR-eQTLs in prostate cancer, genome-wide germline genotype data (Affymetrix 6.0) were obtained for prostate cancer cases from The Cancer Genome Atlas (TCGA). Following genotyping quality control, imputation of non-genotyped variants, we further obtained tumour tissue miRNA-sequencing data from the same set of prostate cancer patients. We performed miRNA alignment and expression quantification adding recently reported novel miRNAs data to the analysis. Linear regression models were used to identify potential miR-eQTLs using the MatrixEQTL R package. We observed several recently reported novel miRNA genes whose expression was significantly associated ($P < 0.05$, false discovery rate (FDR) < 0.1) as well as known miRNAs with germline variants in primary tumour samples. Moreover, the results demonstrated miR-eQTLs for isoforms of known/novel miRNAs in addition to miR-eQTLs regulating the known/novel miRNAs that carry one mismatch. The results have highlighted the potential of exploiting miR-eQTLs as a tool to map new key miRNA genes involved in prostate

cancer, which ultimately could lead us to discover networks of dysregulated miRNAs contributing to prostate tumourigenesis.

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P12.106C

Variant profiling of colorectal adenomas from patients with *MSH3*-related adenomatous polyposis

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Aim: Biallelic *MSH3* germline mutations cause a rare subtype of adenomatous polyposis. In the present study we comprehensively analyzed the variant spectrum of colorectal adenomas from patients with biallelic *MSH3* germline mutations to identify potential driver genes and pathways of MSH3 related tumorigenesis.

Material and Methods: We performed whole exome sequencing (WES) and array-based copy number variant (CNV) analysis of 2-3 adenomas and matched normal tissue in three patients with biallelic *MSH3* mutations.

Results: The amount of all somatic variants in the MSH3 adenomas (36-120) and the pattern of single nucleotide variants (SNVs) seem to be similar to sporadic adenomas, whereas the fraction (16-40%) of small insertions/deletions (Indels) is higher. Interestingly, pathogenic somatic *APC* mutations were found in all adenomas, and the vast majority (6 of 7) are Indels, which seem to occur more often (4 of 6) in short nucleotide tandem repeats compared to published somatic and germline *APC* Indels. On average, 26 large deletions were found in each of three fresh-frozen adenomas per patient. Besides *APC*, five more genes harbor truncating mutations, missense variants, or deletions in more than one polyp, including the suggested tumor suppressor gene *ELF3*. A second hit was only found in *APC*.

Conclusions: Our preliminary data demonstrate that MSH3-related carcinogenesis seem to follow mainly the classical APC-driven pathway. We found similar mutation patterns of SNVs in MSH3-deficient polyps compared to sporadic adenomas, however, in line with the specific function of MSH3 in the mismatch repair system, we observed a high proportion of Indels.

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P12.108A

Ruxolitinib regulates mechanisms of apoptosis and autophagy in multiple myeloma cells

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Multiple myeloma is distinguished by the aggregation of differentiated plasma cells primarily in the bone marrow. In this study, we investigated the genetic basis of apoptosis and autophagy co-regulation upon ruxolitinib treatment in multiple myeloma cells ARH-77 and healthy B lymphocyte NCI-BL2171 as a control group. Cell lines were incubated and cultured with optimal growth conditions. Ruxolitinib dissolved in DMSO. The cytotoxic activity was determined using the WST1 cell proliferation test. IC_{50} doses were calculated in a time and dose-dependent manner. The Annexin V-FITC detection kit and Premo autophagy Tb/GFP TR-FRET LC3B expression kit were used for apoptosis and autophagy analyses, respectively. Gene expression changes were determined using real-time PCR method and analyzed by $2^{-\Delta\Delta Ct}$ method. IC_{50} doses of ruxolitinib in ARH77 and NCI-BL2171 for 72 hr incubation period was found as 20,03 uM and 23,6 uM, respectively. Ruxolitinib treatment induced apoptosis in NCI-BL2171 and ARH-77 cell lines by 6.5 and 2 fold, respectively. The agent increased the autophagic flux by 1.7 and 3.45 fold in NCI-BL2171 and ARH-77 cell lines, respectively. The expression levels of APAF1, BIK, CASP6, TRAF2 genes which regulate mechanisms of apoptosis and autophagy were upregulated 12.07, 4.06, 2.81, 2.77 folds, respectively. Our results indicate that JAK2 inhibitor ruxolitinib regulates apoptosis and autophagy pathways simultaneously in multiple myeloma cell line, ARH77. This regulation is further confirmed by analysis of critical gene expressions which paves the potential of the agent as a therapeutic for

multiple myeloma. Our study is supported by Ege University Scientific Research Projects Coordination.

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P12.109B
Mutation Pattern in Periampullary Carcinoma in Indian Patients

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Introduction: Periampullary carcinoma (PAC) is a heterogeneous group of cancer and genetic abnormality is one of the major risk factor associated with prognosis and survival of patients with this cancer. There are no studies on mutation profile from India in PAC. We present mutation profile of 60 PAC in Indian patients.

Materials and Methods: Sixty cases of PAC were reviewed histologically. DNA was extracted from paraffin tissues and ultra deep sequencing was done using Ampliseq cancer hotspot panel v2 in tumour and normal tissue samples. The results were analysed on ion-reporter software version 5.10.

Results: 30/60 (50%) cases of PAC showed high grade tumor histology (moderate/poor differentiation). KRAS, CTNNB1, STK11, RET, HRAS, EGFR, ATM, NOTCH1 and AKT1 gene mutations were found in 43%, 20%, 16%, 16%, 10%, 10%, 10%, 6.6% in high grade tumor, whereas mutation of these gene in low grade tumorwas26%, 10%, 6.6%, 6.6%, 6.6%, 6.6%, 3.3%, 3.3% respectively. Three different actionable mutations were found in CTNNB1 mutant cases.PIK3CA mutation frequency was seen in 30% of low grade tumor and 13.3% high grade tumor.

Conclusion: KRAS, CTNNB1, STK11, RET, HRAS, EGFR, ATM, NOTCH1 AKT1 and PIK3CA that are involved in different signalling pathways were found to be frequently mutated in PAC indicating their role in disease development and progression. Actionable mutation KRAS, PIK3CA, CTNNB1, EGFR, NRAS and TP53, were found in 36/60 (67%) cases which may benefited in future through targeted therapy.

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P12.110C
Genetic analysis of neuroblastoma in African-American children

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Neuroblastoma (NB), a pediatric cancer with a high degree of clinical heterogeneity, is rarer in African-American (AA) children compared to children of European descent. AA children with NB, however, more frequently develop the high-risk form of the disease and have associated lower overall survival. We have identified several loci associated to NB by GWAS performed in children of European descent. In this study, we have genotyped 674 AA NB cases and 3113 AA controls to investigate genetic susceptibility to NB in this population. Following high density genome-wide imputation, we were able to confirm one susceptibility gene (BARD1), which reached genome-wide significance in the subset of high-risk cases. Polygenic score analysis based on significance and estimates of SNP effect sizes from the European-American (EA) GWAS, detected a highly significant association ($p = 2.3x10^{-14}$) with a score which included all SNPs with $p < 5.5x10^{-7}$, and explained ~3% of NB risk variance in AAs. However, the significance of the polygenic score dropped rapidly with inclusion of additional SNPs, suggesting either limited sharing of NB genetic risk factors between EAs and AAs, or a genetic architecture of NB with limited contribution from common SNPs. Other genetic analyses (including admixture mapping and haplotype association analysis) are in progress to test whether other NB susceptibility variants are located in regions of the genome that show different genetic ancestry in AA cases versus controls. These in particular may help explain susceptibility to developing the high-risk form of NB that disproportionately affects AA children with NB.

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P12.111D
Constitutional mismatch repair deficiency as a differential diagnosis of neurofibromatosis type 1: consensus guidelines for testing a child without malignancy

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Introduction: Constitutional mismatch repair deficiency (CMMRD) is a rare childhood cancer predisposition syndrome caused by bi-allelic germline mutations in one of four mismatch-repair genes. Besides very high tumour risks, CMMRD phenotypes are often characterized by the presence of signs reminiscent of neurofibromatosis type 1 (NF1). Because NF1 signs may be present prior to tumour onset, CMMRD is a legitimate differential diagnosis in an otherwise healthy, suspected NF1/Legius syndrome child without a detectable underlying *NF1/SPRED1* germline mutation. However, no guidelines indicate when to counsel and test for CMMRD in this setting.

Methods: At an interdisciplinary workshop, we discussed estimations of the frequency of CMMRD as a differential diagnosis of NF1 and potential benefits and harms of CMMRD counselling and testing in a healthy child with no malignancy. Assuming that CMMRD is rare in these patients and that expected benefits of identifying CMMRD prior to tumour onset should outweigh potential harms associated with CMMRD counselling and testing in this setting, we elaborated a strategy to pre-select, among suspected NF1/Legius syndrome children without a causative *NF1/ SPRED1* mutation and no overt malignancy, those children who have a higher probability of having CMMRD. Pre-selection criteria and strategies for counselling and testing were developed and reviewed in two rounds of critical revisions.

Results and Conclusions: Existing diagnostic CMMRD criteria were adapted to serve as a guideline as to when to consider CMMRD as differential diagnosis of NF1/Legius syndrome. In addition, the guidelines include counselling and testing strategies that are suggested to minimize potential harms.

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P12.112A
Validation and utilization of NGS based HEMEaccuTest™ panel and analysis software for Hematological malignancies

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Hematological malignancies are forms of cancer originated in blood-forming tissue or in the immune cells and can be classified as leukemias, lymphomas, multiple myeloma, myelodysplastic syndromes, and myeloproliferative neoplasms. Detecting genetic alterations in hematological malignancies is important because of diverse variants associated with classification and subtype diagnosis, and prognostic and therapeutic prediction. NGS is an advanced technology to simultaneously analyze multiple genes to identify clinically well-described variants, as well as rare variants related to hematological malignancies for clinical diagnostics. HEMEaccuTest™ is a targeted NGS panel for hematologic malignancies and NGeneAnalySys™ is automatic analysis software for estimating the relative pathogenicity of detected variants. HEMEaccuTest™ covers entire exon regions of 108 genes putatively known for associations with the diseases according to the WHO, NCCN and ELN guidelines. The diagnostic utility of the panel and software were validated using genotype-known references and clinical specimens. The results showed that pathogenic variants were effectively detected with an average coverage depth of 600x and a minimum coverage depth of 100x. It demonstrated an excellent limit of detection, with 100% sensitivity for SNVs at 2% VAF and for indel at 4% VAF. In addition, the analytical sensitivity and specificity of the panel were high in a comparison to conventional methods such as Sanger

sequencing. Noticeably, the approximately 300-bp-size insertions of FLT3-ITD was detected by a simulating algorithm of NGeneAnalySys™. Thus, this analytical validation demonstrated that HEMEaccuTest™ and NGeneAnalySys™ can be an excellent tool for disease definition and therapeutic strategy in hematological malignancies.

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P12.113B
Clinical applicability of NGS panel in hereditary cancer

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Introduction: Next-generation sequencing (NGS) allows to concurrently analyze germline variants of multiple genes associated with hereditary cancer syndromes.

Methods: 3118 DNA samples of oncologic patients (2177) and healthy relatives (941) from families with cancer history fulfilling clinical criteria were analyzed using NGS panel CZECANCA (CZEch CAncer paNel for Clinical Application) [1]. The most common types of the patients´ cancer were: breast (62.6%), ovarian (12.6%), colorectal (9.2%). This panel targets 226 genes based on the genetic variability of Czech cancer patients. We evaluate 78 clinically relevant genes. For CNV (copy number variation) detection we use sequencing coverage analysis.

Results: In total samples 18,6% a deleterious variants (class 4-5) were detected- in 20 % of patients with breast cancer, in 21,9 % of patients with ovarian cancer and in 21,4 % of patients with colorectal cancer. In case of reporting only variants in genes recommended by clinical guidelines or genes covered by commercially available oncopanels the detection rate would decrease to 13%. Within CNV validation, we tested 90 findings in 1175 individuals by MLPA. 29 (32.22%) were true positive (TP), the remaining 61 samples (67.78%) were false positive (FP). Costs in this settings dropped by 3/4 in comparison with MLPA testing only.

Conclusion: These results are consistent with the published findings and highlight the importance of extending the examination to other susceptibility genes which brings more information to the patients. This allows more precise genetic testing and genetic counselling in families followed by preventive care.

[1] Soukupova et al., 2018, https://doi.org/10.1371/journal.pone.0195761

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P12.114C
Transmembrane nodal complex is abnormal in NOMO-1 deleted colon cancer

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Introduction: *NOMO-1* (located in 16p13.1) somatic deletion has been proven in recent studies to occur in most cases of early-onset, non-hereditary, microsatellite-stable colorectal cancer. However, the role of this alteration in carcinogenesis is unknown. Thus, the main aims of this study are to generate a stable colorectal cancer cell line that does not express this gene and to analyse the nodal pathway in this line.

Materials and Methods: CRISPR-Cas9 technology was used to generate a *NOMO-1* knockout from the colorectal cancer-derived cell line HT29. The efficacy of its design was tested by Sanger sequencing, quantitative polymerase chain reaction (qPCR) and western blot. To study off-target effects, chromosomal differences were measured by CytoScan genomic microarrays platform. Changes in Nodal pathway protein expression were analyzed by western blot.

Results: The *NOMO-1* knockout cell line's design efficacy was proven. However, the genomic microarrays showed a complete deletion of chromosome 16. Nodal pathway protein expression remained unchanged except for severe downregulation of TMEM147 and Nicalin. Both proteins are part of a transmembrane complex with NOMO.

Conclusions: NOMO transmembrane complex has been reported to be an antagonist of the nodal pathway. Our results show that NOMO transmembrane complex is absent in *NOMO-1* knockout cancer cells. However, downstream proteins remain unaltered in these cells, suggesting that NOMO could regulate another signaling pathway in adult cancer cells. Further studies would be required for

completing the functional characterization of *NOMO-1* deletion in early-onset colorectal cancer.

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P12.115D

Genetic study of the Nodal signaling pathway genes in colorectal cancer

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Introduction: Recent studies show the carcinogenic impact of the Nodal pathway, which is upregulated in certain types of tumors and has been linked to tumorigenesis and metastasis in melanoma, breast, colon, ovarian, prostate, endometrial and pancreatic cancers. Its inhibition decreases tumorigenesis. We performed a genetic study of the genes relevant to this pathway, as well as their potential inhibitors in colorectal cancer (CRC).

Materials and Methods: A specific Illumina panel was designed by our group to study the Nodal pathway genes and inhibitors. This panel includes the following genes: *ALK4 (ACVR1B), ACTRII, SMAD3, NCNL, SMAD4, NOMO1, SMAD2, TEMEM147, LEFTY, GPR78, TDGF1*. A total of 36 CRC were studied via sequencing in a MiSeq. The results were analyzed with VariantStudio (Illumina) and IGV 2.4 software.

Results: A total of 20 pathogenic nonsense mutations were identified in 19 of the 36 analyzed tumors; 9 of which (45%) were found in the *NOMO1* gene generating a truncated protein. Additionally, 4 pathogenic mutations were found in *SMAD3*, 3 in *SMAD2*, 2 in *SMAD4* and only one mutation was found in *NCLN* and *ALK4*.

Conclusions: A high percentage of CRC show mutations in one of the Nodal pathway genes. Due to the high number of mutations found, the NOMO-mediated signaling could be implicated in the tumorigenesis of CRC. This work was supported by a grant from Instituto de Salud Carlos III (Ministry of Economy and Competitiveness) (ISC III-FEDER: PI16/01920).

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P12.116A

Discovery of human mir-1839: a miRNA derived from a snoRNA/scaRNA

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Small nucleolar RNAs are a class of small non-coding RNAs involved in the pseudouridylation and methylation of ribosomal RNAs. A subclass of snoRNAs, called Small Small Cajal body-specific RNAs perform the same chemical modification of spliceosomal RNAs U1, U2, U4, U5 and U12. Previously, it has been shown that some microRNAs can be a derivative of snoRNAs. By a small RNA-seq experiment of total RNA from a uterus adenocarcinoma, we identified a novel human mature microRNA that is identical to the microRNA mmu-miR-1839-5p. Upon alignment of the sequence of hsa-miR-1839-5p, we noticed that its mature sequence is embedded in the same direction within a gene encoding a human scaRNA (scaRNA15/ACA45), within the long arm of chromosome 15q25.2. We designed an *in vitro* stem-loop RT-PCR assay in order to validate the existence of the novel species of microRNA in the tissue where it was discovered and also to evaluate expression level in a number of total RNAs extract of different adult, fetal and neoplastic tissues. The stem-loop RT-PCR assay revealed that hsa-miR-1839-5p is more expressed in two neoplastic tissues in comparison with their non-neoplastic counterparts. Additionally, the novel human microRNA is expressed in widely variable degrees in a variety of 9 more adult total RNAs as well as 5 total fetal RNAs.

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P12.117B

User-friendly and machine learning-empowered platform for classification of NSCLC based on RNA-seq profiling

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Introduction: RNA-seq has become a standard method for transcriptome profiling of cancer tissues. A great number of studies investigated genetic expression in order to identify marker genes that could be used for diagnostic and/or prognostic purposes. However, the generalisation of expression pattern with limited number of genes may be inaccurate. Instead of using a specific subset of genes, we propose to use the whole RNA-seq profile to improve the diagnosis and classification of Non-Small-Cell Lung Cancer (NSCLC) through a user-friendly platform.

Materials and Methods: A machine-learning algorithm (Random Forest) which learns from RNA-seq profiles of NSCLC tissues, collected in 123 patients (tumour and non-tumour samples) was applied in order to discriminate histological subtypes of NSCLC. The method has been implemented within the R environment and the web platform has been built with the Shiny package.

Results: The web platform actually allows the user, first, to provide its own RNA-seq library; then it automatically fits a Random Forest model based on the expressed genes; finally, it returns a diagnosis along with its false discovery rate. The prototype was assessed and cross validated. The best results were obtained when full RNA-seq profiles were provided.

Conclusions: We propose our method as a universal approach of use of whole RNA-seq data for diagnostic purposes in a simple and straightforward manner.

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P12.118C
Circulating nucleoprotein complexes for breast cancer patients are enriched by tetratricopeptide-like and ion channel proteins

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Introduction: Cell-free DNA (cfDNA) circulated in bloodstream being packed into membrane coated structures or forming deoxyribonucleoprotein complexes (DNPCs).

Materials and Methods: DNPCs were isolated by affinity chromatography with antihistone antibodies, the proteins were identified by MALDI-TOF. Studying the protein content in DNPCs circulating in the blood plasma of ten healthy females (HFs) vs primary breast cancer ten patients (BCPs) by analysis of protein signatures & GO annotation.

Results: HFs: 176 proteins joined with 195 GO terms were characterized; BCPs: 167 proteins joined with 168 GO terms were characterized; only 38 proteins (12% of 305 different proteins) & 96 GO (40% of 267 different GO) are common for both HFs and BCPs. Our results assumed extracellular protein fragments are involved in binding with cfDNA for BCPs over HFs states for two protein pools. Only for BCPs we have identified DNPCs formed by 5 tetratricopeptide-like helical domain superfamily proteins (P61201, Q49AM3, Q6PGP7, Q92623, Q96EK5), and 5 ion channels (P48167, P48995, Q6PIU1, Q8NEC5, Q96KK3). It allows suggest the reason for the increase of cfDNA concentration in the blood during the development of tumors. In according GO analysis (cellular component) membrane proteins are more represented in the DNPC composition, which suggests the binding of circulating DNPC with the cell surface.

References: [1]Tamkovich SN, et al (2015). Identification of proteins in blood nucleoprotein complexes. doi:10.1134/S1068162015060163; [2]Tamkovich SN, et al (2016). Protein Content of Circulating Nucleoprotein Complexes. doi:10.1007/978-3-319-42044-8_26. [3]Belenikin MS, et al (2018). Protein composition characterization of circulating nucleoprotein complexes. ESHG-2018 poster: E-P16.03.

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P12.119D
Significance of variants of the unknown in clinical practice of oncogenetics

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Introduction: Advancements in genetic techniques lead to increasingly more sequence information. However, many of novel sequence variants are not meaningful in clinical decision-making and are referred as -,the gray zone". Our study objective was to review the distribution of variants of unknown significance (VUS) in high risk breast and ovarian cancer patients.

Materials and Methods: 499 female patients with increased risk (young age and/or positive family history and/or triple negative breast cancer) of familial/hereditary breast or ovarian cancer were included in this study. The mean age of patients was 53.3±9.6. Genetic counseling and NGS analysis were performed at the Klaipeda University Hospital (Lithuania) from 2015 until 2018. Mutations were detected using Next Generation Sequencing (Ion Torrent™ PGM) custom Ion AmpliSeq™ On-Demand panel (Thermo Fisher Scientific). Custom oncogene-panel was made of forty-four genes, associated with familial hereditary and syndromic cancer diseases.

Results: Mutations were detected in 181 of 499 (36%) samples. Pathogenic mutations consisted 61% (111) of all detected mutations. High frequency of pathogenic mutations is explained by targeted study group of cancer patients. 7 (4%) patients had a combination of pathogenic mutation and VUS. VUS was detected in 52 (29%) patients.

Conclusions: Our results show that VUS remains an important problem in the clinical interpretation of high-risk cancer patients analysis results. Thus, it is of paramount importance in diagnostic studies to classify mutations according to the status - pathogenic or benign, and to update biological databases.

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P12.120A

Study the role of miR34 family and miR203a in the diagnosis of ovarian cancer

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Introduction: Ovarian cancer is the fifth most common form of cancer death among women, due to the difficulty of diagnosis. Circulating miRNAs proved to be promising biomarkers in various cancers. However, only few publications focus on circulating miRNAs in ovarian cancer especially in European populations.

Materials and Methods: We screened the members of the miR34 family (miR34a, miR34b, miR34c) and miR203a in the plasma samples of patients with malignant (n=28, I, III or IV FIGO stage), non-malignant (n=12) ovarian tumors and in age-matched healthy volunteers (n=60). The relative amount of miRNAs was detected by qPCR.

Results: The expression levels of miR34a, miR34b and miR203a were higher in the malignant samples compared to the healthy donors ($p<0.05$; 0.001; 0.001 respectively). However, no significant difference was detected in the case of miR34c. Diagnostic accuracy was the highest in the case of miR203a: 84.09% with the AUC of 0.831 (95% CI=0.727-935). Spearman's rank correlation revealed positive correlation between the expression values of miR34s and miR203a that was the highest between miR34a and miR34b. The agreement between the diagnostic tests based on miR34s and miR203a proved to be good according to the Cohen's kappa values with the highest value between miR34a and miR34b. However, the diagnostic tests based on these miRNAs and the standard CA125 and HE4 showed low agreement. Target analysis revealed that miR34a and miR34b share several target genes involved in cancer development.

Conclusions: We conclude that miR203a and miR34b might be promising complementary markers of CA125 and HE4 in ovarian cancer.

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P12.121B

Germline pathogenic variants of cancer susceptibility genes among Japanese ovarian cancer patients

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Aim: The aim of our study was to reveal the prevalence of pathogenic germline variants of candidate genes associated with genetic predisposition to OC in Japanese OC patients.

Method: Germ-line DNA samples from 230 unselected OC patients were recruited from the Keio Women's Health Biobank at Keio University School of Medicine. Germ-line DNA was enriched using the SureSelect XT Target

Enrichment System (Agilent Technologies) designed for 75 or 79 genes as a custom OC panel, followed by sequencing using MiSeq (Illumina). Detected variants were classified according to the American College of Medical Genetics and Genomics recommendations. Furthermore, BRCA1/2 variants were interpreted using resources from Myriad Genetic Laboratories.

Results: Of 230 patients, 19 (8.3%) and 8 cases (3.5%) carried germline *BRCA1* and *BRCA2* pathogenic variants, respectively. No variant of uncertain significance (VUS) of *BRCA1/2* genes was detected in our analysis according to the database of Myriad Genetics. Six (2.6%) carried pathogenic germline variants of mismatch repair genes. Carriers of *BRCA1/2* or pathogenic variants of any other genes tested were more likely to be diagnosed younger, have first or second-degree relatives with OC, and have OC classified as high-grade serous carcinoma (HGSC).

Conclusion: Our data can facilitate genetic predisposition prediction in Japanese OC patients and referring high-risk patients for genetic counseling and testing.

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P12.122C
The influence of *BIRC5* polymorphisms and GLI proteins on the expression of survivin isoforms in ovarian cancer

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Introduction: Ovarian cancer (OC) is one of the most lethal cancers, mostly due to late diagnosis and limited treatment options. Survivin (coded by *BIRC5* gene) is an inhibitor of apoptosis, has at least five different splice variants and is often overexpressed in cancer. Survivin is direct target of Hedgehog-GLI signaling pathway, a major developmental pathway involved in organogenesis and stem cell maintenance.

We investigated the role of *BIRC5* polymorphisms and survivin isoform expression in OC. Additionally we investigate the role of different GLI proteins in the regulation of survivin isoform expression in the SKOV-3 OC cell line.

Materials and Methods: 40 OC samples and 74 controls were genotyped for *BIRC5* polymorphisms. Survivin isoform expression was analyzed on 29 OC samples and compared to healthy fallopian tube (FT) controls. To investigate the regulation of survivin expression by GLI proteins, GLI knock-outs were developed and survivin expression was determined.

Results: Fifteen different polymorphisms were found in OC samples, and there was no difference in distribution between OC samples and controls. Several polymorphisms showed linkage disequilibrium in OC samples. All five isoforms were detected in OC samples, and only two isoforms in FT controls. Seven polymorphisms showed significant associations with isoform expression. Survivin isoforms were downregulated in GLI1 and GLI2 knock-outs, but not in the GLI3 knock-out. Treatment of GLI1 knock-out with GANT-61 showed an additional inhibitory effect on several isoforms.

Conclusions: Survivin isoform expression is regulated by both *BIRC5* polymorphisms and the expression of GLI transcription factors that bind to its promoter.

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P12.123D
Circulating miR200 family members are promising candidate biomarkers in the diagnosis of ovarian cancer

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Introduction: Ovarian cancer is the most lethal form of gynecological malignancy. CA125 and HE4 are frequently used biomarkers in ovarian cancer, however, these have low diagnostic parameters. Here we studied the diagnostic potential of circulating miRNAs in ovarian cancer.

Materials and Methods: We screened the members of the miR200 family (miR200a, miR200b, miR200c, miR141, miR429) in the plasma samples of patients with malignant (n=28, I, III or IV FIGO stage) or non-malignant (n=12) ovarian tumor and in age-matched healthy controls (n=60). The relative amount of miRNAs was detected by qPCR.

Results: All the tested miRNAs showed significantly elevated expression in the malignant samples compared to the healthy controls ($p<0.001$). Moreover, the relative expression of miR200a, miR141 and miR429 proved to be higher in the malignant than in the non-malignant samples ($p<0.05$). ROC-AUC proved to be the highest in the case of miR200c: 0.861 (95% CI=0.776-0.947). Spearman's rank correlation analysis revealed positive correlation between the plasma levels of the studied miRNAs that was the highest between miR200b and miR200c ($r_s = 0.775$;

p<0.001). Target analysis also suggested tight interaction between these miRNAs in the regulation of cancer development. The agreement of diagnostic tests based on miRNA levels and the standard CA125 or HE4 was weak according to Cohen's kappa values.

Conclusions: MiR200 family members might be promising complementary biomarkers in the diagnosis of ovarian cancer. The plasma level of tightly interacting miRNAs shows strong positive correlation. Grant reference: NTP-NFTÖ-18-B-0377

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P12.124A
Study of DNA repair gene polymorphisms in ovarian cancer

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Introduction: Ovarian cancer (OC) is proven to be influenced by alterations in some DNA repair genes (e.g. BRCA1/2). In our work, we analyzed in DNA repair genes polymorphisms (SNPs) by TaqMan genotyping in OC peripheral blood samples.

Material and methods: An association study of SNPs rs1799782, rs25487, rs1130409, rs13181, rs11615, rs1799794, rs861539, rs1042522, rs1799977 and rs1800734 of genes *XRCC1, APEX1, ERCC2, ERCC1, XRCC3, MLH1* and *TP53* was performed in the germinal DNA of 185 patients and 129 healthy controls.

Results: the GA genotype of *XRCC1* polymorphism rs1799782, the CC genotype of *TP53* polymorphism rs1042522 and the GG genotype of *MLH1* polymorphism rs1800734 were associated with increased susceptibility to OC. The T allele of *APEX1* polymorphism rs1130409 was associated with a later onset of the disease and hereditary OC. In relation to *XRCC3* gene (rs1799794), the TT genotype was associated with increased OC susceptibility in aging patients and patients with familial OC. In relation to *MLH1* gene (rs1800734), the GG genotype was associated with greater OC susceptibility in old patients and in patients with hereditary OC. The SNP rs1799977 of *MLH1* gene was associated with an increased risk of recurrence.

Conclusions: Our study suggests that DNA repair genes different from *BRCA1/2* like *XRCC1*, *TP53*, *MLH1*, *XRCC3* and *APEX1* could modify the risk of developing OC. This project was funded by FIS-FEDER PI16/01920.

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P12.125B
Identification of differentially expressed miRNAs in plasma samples of ovarian cancer patients

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Introduction: Ovarian cancer (OC) is the 6th most common tumor in women. Its high mortality rate is partially due to lack of effective screening methods for early diagnosis. Given their ability to regulate gene expression and presence in bio-fluids miRNAs could be non-invasive biomarkers for cancer diagnosis.

Materials and Methods: Total RNA was isolated from plasma samples of 18 OC (6-6 FIGO stage I, III and IV) patients and 6 healthy controls. MiRNA copy number was determined using the Nanostring System with nCounter Human v3 miRNA Panel. Background and technical variations were corrected for the mean±2SD of negative controls and the positive code-set, respectively. Data was normalized by the geometric mean of 10 housekeeping miRNA counts. Significant differentially expressed (DE) miRNAs were identified by Kruskal±Wallis test with *post hoc* Dunn's test. Target genes, candidate pathways and lncRNA associations for significant miRNAs were identified by a network-based analysis using miRNet, mirTargetLink and Networkanalyst tools. Functional annotation clustering of target genes and pathway analysis was done with the DAVID tool.

Results: 26 miRNAs were upregulated, the tumor suppressor miR-584-5p was down-regulated. In our DE miRNA set miR-25-3p/26b-5p/301a-3p/19b-3p/144-39 had the highest degree and betweenness centrality values, their

common targets are PTEN, BCL2L11, KAT2B, SMAD4, TP53, MALAT1, XIST, HOTAIR.

Conclusion: Functional annotation and gene-GO term enrichment analysis of targets identified the involvement of cell cycle regulation, FOXO/PI3-AKT/TP53/TGFβ/SMAD4 signaling pathways, negative regulation of apoptosis, positive regulation of proliferation and epithelial-mesenchymal transition in OC development. Specific plasma miRNA profiles could represent potential diagnostic biomarkers for OC.

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P12.126C

Combination of germline and tumor testing yields a high rate of loss-of-function variants in non-mucinous epithelial ovarian cancer patients

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Non-mucinous epithelial ovarian cancer diagnosis is a stand-alone criterion for genetic testing referral, irrespectively of family history or age at diagnosis. Identification of at least *BRCA1 & BRCA2* mutations are fundamental for clinical decision making of ovarian cancer (OC) patients. We analyzed genomic DNA from 578 epithelial non-mucinous OC patients for mutations, implementing a commercially available 94-gene panel. Additionally, 121 tumors were collected from OC patients with negative germline testing and assessed for somatic *BRCA1 & BRCA2* mutations. Overall, 25.4% (147/578) of the patients carried germline loss-of-function (LoF) variants, distributed in 18 genes. After *BRCA1 & BRCA2*, which accounted for 72.1% of the total, *RAD51C* LoF variants were the most frequent (4.8%). Interestingly, the vast majority of LoF variants (136/147; 92.5%) involved homologous recombination/Fanconi anemia genes, while 4.5% of the pathogenic variants were detected in Mismatch Repair (MMR) pathway genes. Subsequently, tumor analysis resulted in the identification of damaging *BRCA1 & BRCA2* variants in 11.5% of the ovarian tumors tested. Variant allele frequencies varied from 9%-47%. Altogether, approximately one in three of OC patients in our cohort could be good candidates for therapies targeting defective mechanisms of DNA repair, including HR and MMR. Our study highlights the high prevalence of LoF variants in HR genes, when combining germline and tumor testing in unselected, non-mucinous epithelial OC patients. These genetic defects can predominantly lead to HR deficiency, the ultimate biomarker for therapeutic intervention by PARP inhibition leading to tumor-cell death.

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P12.127D

Pathogenic germline mutations in *BRCA1, BRCA2* and *CHEK2* in a single patient with pancreatic acinar cell carcinoma

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Here we report on a family with hereditary breast and ovarian cancer with a known BRCA1 frameshift mutation (NM_007294.3:c.5266dupC, p.(Gln1756Profs*74)). The index patient's son was diagnosed with pancreatic acinar cell carcinoma at 34 years of age soon after the molecular testing in the family. Pancreatic acinar cell carcinoma is a very rare tumor with a poor prognosis and limited treatment options. He was therefore recommended testing of the tumor tissue with regards to possible targeted therapeutic options in the future (PARB-inhibitor therapy).

Using a multi-gene panel we surprisingly not only identified the familial BRCA1 mutation in the tumor tissue in heterozygous state but additionally found a frameshift mutation in BRCA2 (NM_000059.3:c.3708dupA, p.(Ala1237Serfs*6)) in 50% of reads as well as a well-known CHEK2-founder mutation (NM_007194.3:c.1100delC, p.(Thr367Metfs*15)) in 50% and an ATM stop mutation (NM_000051.3:c.2426CG, p.(Ser809*)) in 23% of reads. The mutations in BRCA1, CHEK2 and BRCA2 could all be confirmed to be germline mutations, while the ATM mutation was of somatic origin. Of interest the patient's pedigree showed a single case of breast cancer in his deceased paternal grandfather at 60 years of age that had not further been investigated.

This case challenges the current concept of targeted testing in hereditary cancer predisposition families, especially when cancer predisposition is evident in two branches of the family tree. It also poses the question of adequate clinical surveillance and predictive testing algorithms in a family with three heterozygous pathogenic variants in genes encoding for proteins that are involved in DNA repair mechanisms.

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P12.128A

Analysis of patients with a personal and/or family history of pancreatic cancer with a custom designed broad cancer predisposition gene panel

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Pancreatic cancer is estimated to have a familial background in 5-10% of the cases. Although the underlying genetic basis for most of the familial clustering remains elusive, several familial cancer syndromes are associated with an increased risk of pancreatic cancer.

In this retrospective study, 433 individuals were selected because of a personal or family history of pancreatic cancer in combination with breast and/or ovarian cancer, colon cancer or melanoma.

Germline DNA was analyzed using a custom designed SeqCAP Target panel of 66 (pancreatic) cancer susceptibility genes (Roche).

Data analysis is still ongoing, but we found in 21% of the patients analyzed till now, at least one heterozygous loss of function germline mutation in one of the 66 (pancreatic) cancer susceptibility genes.

As this is a high proportion of the patients, further extension of the study cohort is certainly warranted. In parallel a matched control cohort is being analyzed to determine which genes are significantly more implicated in (pancreatic) cancer predisposition. Further segregation analysis in the families is indicated to evaluate their link with the different cancers and highlight the need for recommendations governing germline multi-gene panel testing of cancer patients with a personal or family history of pancreatic cancer.

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P12.129B

Detection of genome-wide copy number alterations in tumor tissue and cell-free DNA of pancreatic cancer patients

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Diagnosis of pancreatic cancer is very challenging since early stage pancreatic cancer is associated with non-specific and vague symptoms. Additionally, the current diagnostic tools by imaging and CA19-9 measurement have limitations. Liquid biopsies make it possible to detect tumor-specific molecular alterations by analysis of cell free DNA (cfDNA) isolated from plasma, which contains circulating tumor DNA (ctDNA). We evaluated the prevalence of large genomic rearrangements in pancreatic cancer resections and cfDNA of the patients. We applied shallow whole genome

sequencing (sWGS) to cfDNA samples from all patients at different time points. Concordance of the profiles was evaluated between cfDNA and matched FFPE tumor tissue in samples of operable patients. Analyses were finished for 16 operable patients. Clear copy-number alterations (CNAs) were observed in 13 FFPE tumor samples (81%). The profiles were patient-unique but some recurrent alterations were ascertained. In cfDNA of none of the operable patients CNAs were established. In cfDNA of 75% (6/8) of the metastatic pancreatic cancer patients, clear CNAs were identified. In a next step, we are evaluating if this approach can predict recurrence. Indeed, a copy-number change in a follow-up sample (6-months post-adjuvant chemotherapy) was observed in a borderline-operative patient with metastases. These results demonstrate CNAs in cfDNA of advanced pancreatic cancer cases and the potential use of cfDNA derived profiles to monitor treatment response. Additional patients are currently being analyzed and will be presented at the meeting. Research project realized with the support of "Kom op tegen Kanker"

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P12.130C
Peutz-Jeghers syndrome and the spectrum of molecular alterations in the STK11 gene in Bulgaria

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Introduction: Peutz-Jeghers Syndrome (PJS, OMIM 175200) is an autosomal dominant hereditary polyposis syndrome. Clinical features include hamartomatous polyps, mucocutaneous pigmentation, and an increased risk for the development of gastrointestinal (GI) and various extra-GI malignancies. Serine-Threonine Kinase 11 (*STK11/LKB1*) variants predispose to PJS.

Materials and Methods: In this study, we used direct sequencing, followed by MLPA of genomic DNA from 5 patients, clinically and histologically suspected for PJS. Four of the patients carried out known disease causing aberrations, while in one of the patients we found a mutation which has not been reported in the literature or recorded in mutation databases.

Results: In two of the patients we discovered a heterozygous deletion of the whole *STK11* gene, determined by a 50% decrease in all MLPA probes. The third patient showed a heterozygous deletion of exon 1. The fourth and the fifth patient had similar alterations in exon 7, with patient 4 having an in-frame deletion (c.907_915delATCCGGCAG) and the fifth patient- in-frame duplication (c.907_915dupATCCGGCAG) within the *STK11* gene (GRCh38/hg19).

Conclusions: While all of the patients reported here exhibited symptoms of PJS, the severity of the disease and the accompanying symptoms was not determined by the type of the mutation; even in patients 4 and 5, who had alterations in the catalytic kinase domain of STK11. This suggests that even in-frame alterations in the *STK11* sequence have a profound effect. Our findings are encouraging us to further investigate the relationship between the *STK11* gene defects and the clinical severity of the disorder.

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P12.131D
Somatic MYO5Bmutations in adrenal neural tumors promote cancer progression

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We recently reported novel recurrent germline and somatic mutations in the myosin Vb motor gene (*MYO5B*) in malignant pheochromocytoma/paraganglioma (PCC/PGL), an adolescent/adult counterpart to neuroblastoma arising from the same neural crest origin. Here, we have explored the functional impact of three MYO5B missense mutations (p.L587P, p.G1611S, and p.R1641C) with respect to proliferation, migration and intracellular trafficking *in vitro*. MYO5B mutated cDNA clones (pCMV6-MYO5B Myc-DKK tagged) were generated by site directed mutagenesis and stably transfected into human embryonic

kidney cells (HEK293) and SK-N-AS neuroblastoma cells. All three MYO5B mutants demonstrated a significantly increased proliferation rate compared to MYO5B wild type clones ($p<0.001$). The Scratch-wound assay also indicated an increased migration rate ($p<0.05$) in cells harboring the two somatic variants p.L587P (located in the motor domain) and p.G1611S (located in the tail dilute domain). Moreover, investigating the endocytic recycling pathway by the transferrin assay showed an increased transferrin uptake in p.L587P and p.G1611S mutants, indicating a disturbed intracellular transport. Immunohistochemistry and transcriptomic analysis of 31 primary PCC/PGL tumor cases show a differential expression of MYO5B mRNA and protein levels in some malignant PGLs, as well as a changed subcellular localization of MYO5B in one PGL case harboring an acquired p.G1611S MYO5B mutation. We are currently exploring downstream effects in mutant clones by expression microarrays to identify the proliferation pathways activated by *MYO5B* mutations. Our study uncovers the functional role of MYO5B in proliferation and migration in adrenal neural tumors, which might improve future therapeutic implication.

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P12.132A

Characterization of heterozygous *PMS2* variants in French patients with Lynch syndrome

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Heterozygous germline *PMS2* variants are responsible for about 5% of Lynch syndrome (LS) but their prevalence is most likely underestimated, because of the presence of highly homologous pseudogenes, hampering routine and exhaustive screening. Here, we describe a large series of *PMS2* heterozygous variants identified in French LS patients. Two hundred variants were identified in 195 patients including 114 unique variants classified as class-3/4/5 according to consensus guidelines. Although SNVs and small insertion/deletions were predominant, genomic rearrangements accounted for 18% of the alterations. The c.137G>T variant was observed in 36/195 families, accounting for 18% of the PMS2-LS, but the haplotype analysis failed to show a clear founder effect. The median age at first tumour onset in class 4/5 variant carriers was 49, and the predominantly observed tumours were colorectal cancers (76.4%) followed by endometrial cancers (8.1%). The earliest age at tumour onset was 21 and 9 patients developed colorectal cancer by the age of 31. By now, no genotype/phenotype correlation was detected. Among cases with available family history, only 6.8% of class-4/5 carriers had a family history fulfilling Amsterdam I/II criteria. Tumours from *PMS2* variant carriers exhibited microsatellite instability and loss of PMS2 expression in 96% and 76% of the cases, respectively. These results confirm the high predictive value of tumour loss of PMS2 expression and show that, while *PMS2* variants are mostly detected in families not fulfilling Amsterdam criteria, which supports their lower penetrance, they can nevertheless cause colorectal cancers before 31, which highlights the variability of their penetrance.

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P12.134C

Cooperative Androgen and Thyroid Hormone Signaling Drives Prostate Cancer

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Introduction: Androgens drive prostate cancer through androgen receptor signaling and antiandrogen therapy in numerous forms is still key treatment for prostate cancer (PC). In addition to androgens, thyroid hormones have been shown to promote various cancers including PC. Active form of thyroid hormone Triiodothyronine (T3) acts through interaction with nuclear receptors (TRβ/TRα) and binding proteins in cytosol such as μ-crystallin (CRYM). In this study, we investigated the interaction of thyroid hormone with androgen signaling through its nuclear receptor beta (TRβ1) and cytosolic binding protein (CRYM) in the progression of prostate cancer.

Materials and Methods: Tissue microarray (TMA) containing patient samples together with cell lines derived from metastases were used to inspect molecular mechanisms of thyroid hormone driven tumorigenesis. RNA-seq identified alterations of gene expressions in response thyroid and androgen signalling. Co-immunoprecipitation and yeast two-hybrid were employed to inspect a cross-talk between androgen and thyroid signaling. Therapeutic value of thyroid inhibition was carried out through in vivo studies.

Results: TRβ gene was identified as a common feature in drug-resistant tumors, which predicted metastases, and relapse in PC patients. Thyroid hormone binding protein CRYM antagonized thyroid hormone action. Immunoprecipitation and yeast two-hybrid revealed interaction between androgen receptor (AR) and thyroid receptor (TRβ). Inhibition of thyroid hormone signaling resulted in decreased growth of tumor *in vivo*.

Conclusion: Inhibition of thyroid hormone synthesis and action in combination with androgen ablation therapy might have a therapeutic relevance by deducing free T3/T4 levels that might slow down the disease progression and delay recurrence in patients with PCa.

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P12.136A

Genotype phenotype associations in a cohort of 1404 consecutively ascertained retinoblastoma index cases

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Retinoblastoma (Rb), the most common pediatric intraocular neoplasm, results from inactivation of both alleles of the *RB1* gene. To deepen our understanding of Rb, we performed genotype-phenotype associations in a cohort of 1404 consecutively ascertained Rb patients i.e. 866 unilateral Rbs (URb) and 538 bilateral Rbs (BRb) followed between 2000 and 2017. Main characteristics included age at diagnosis, sex, laterality, familial history, IRC stage and mutational status. Advanced IRC stages D and E were mainly observed (67.1% and 19.4%, respectively). Germline detection rate was 93.4% for BRb and 13.0% for URb. Mutations were scattered all along the coding sequence with a predominance of truncating mutations. Close to 50% of first and second hits occurred in 6 distinct regions but these regions differed according to the 1st or second hit (significant for promoter, exons 1, 2 and 24). Compared to nonsense mutations, risk for bilateral Rb was lower for missense, large rearrangement and in frame splice mutations ($p<0.001$, $p<0.05$ and $p=0.05$ respectively). As compared to germline mutations maintaining pRb, germline mutations inducing the absence of pRb led to an earlier median age at diagnosis (9 months vs 12 months, $p<0.05$), more frequent bilateral involvement (84.1% vs 65.2%, $p<0.001$), and an advanced IRC stage (85.3% vs 73.9%, $p<0.05$). Surprisingly, the opposite was found for 2nd hits where absence of pRb was more often associated with unilateral RB (OR = 46.11, $p<0.001$). This is the largest genotype-phenotype study reported to date, opening new avenues for an in depth understanding of the disease.

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P12.137B
Ruxolitinib regulates energy metabolism of multiple myeloma cells

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Aim: Multiple myeloma is the cancer of bone marrow plasma cells. Ruxolitinib which is a potent inhibitor of JAK tyrosine kinases is approved for treatment of myeloproliferative neoplasms. In this study, our purpose was to determine the possible role of ruxolitinib on energy metabolism of ARH77 multiple myeloma cells which has not been investigated so far.

Methods: ARH77 multiple myeloma cells were incubated via RPMI-1640 medium. Ruxolitinib was dissolved in DMSO. Seahorse XFp Cell Energy Phenotype Test Kit (Agilent) was used to determine the phenotypic effects of ruxolitinib on energy metabolism of ARH77 cells. Changes in expression level of the genes which control energy pathways were determined by Glucose Metabolism RT^2 Profiler PCR Array (Qiagen), RT^2 SYBR Green qPCR Mastermix (Qiagen) and qRT-PCR instrument (LightCycler480, Roche). Fold changes were calculated via $2^{-\Delta\Delta Ct}$ quantitation method.

Results: Ruxolitinib increased oxygen consumption and extracellular acidification rates 1.45 and 1.68 folds compared to control, respectively. Ruxolitinib also regulated expression levels of PYGM, PGM2, PHKB, PCK2, H6PD, PDK4 and GYS2 genes higher than 2 folds, which regulate cell energy metabolism.

Conclusion: The results of our study indicate that ruxolitinib genetically regulates cell energy metabolism which has not been previously evaluated in ARH77 cells, and this change is reflected in cellular phenotype. Further studies which research the potential role of ruxolitinib in energy metabolism will provide a new perspective in myeloma genetics.

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P12.138C
Detection of cell-free, exosomal and whole blood mitochondrial DNA copy number in plasma or whole blood of patients with serous epithelial ovarian cancer

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Ovarian tumor is one of the leading causes of cancer among women. It is usually diagnosed at an advanced stage. In search for new specific and sensitive biomarkers we detected mitochondrial DNA copy number in whole blood (wb-mtDNA) and in plasma (cell-free and exosome encapsulated mtDNA) in patients with serous epithelial ovarian tumor.

DNA was isolated from EDTA blood and plasma obtained from 24 patients and 24 healthy controls. Exosomes were isolated from cell-free plasma, and exosomal DNA was extracted. Quantitative-real-time PCR was performed with Human Mitochondrial DNA (mtDNA) Monitoring Primer Set. Kruskall-Wallis and Mann-Whitney test were used for data analysis.

Wb-mtDNA copy number was significantly different among healthy controls and patients in multiple comparison ($p=0.0090$ considering FIGO stages independently, and $p=0.0048$ considering early- and late-stage cancers). There was a significant decrease among early-stage, all advanced stage and all cancer patients (FIGO I: 32.5±8.3, $p=0.0061$; FIGO III+IV: 37.2±13.7 $p=0.0139$; FIGO I+III+IV: 35.6 ±12.2, $p=0.0017$) or FIGO III patients alone (32.8±5.6, $p=0.0089$) compared to healthy controls (48.6±17.1).

We found significant increase in exosomal mtDNA copy number in cancer patients (236.0±499.0, $p=0.0155$), advanced-stage cancer patients (333.0±575.0, $p=0.0095$), FIGO III (362.0±609.2, $p=0.0494$), and FIGO IV (304.0 ±585.0, $p=0.0393$) patients alone compared to healthy controls (9.3±7.1) and in multiple comparison considering early- and late-stage cancers ($p=0.0253$). Cell-free mtDNA copy numbers were not increased significantly.

We observed significant difference in wb-mtDNA copy number in case of early- and late-stage cancer patients and in exosomal mtDNA copy number in case of late-stage cancer patients compared to healthy controls.

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P12.139D
Generation of Single Cell NGS Library with High Reproducibility, and Greatly Improved Coverage and Fidelity for Precision Medicine

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Introduction: Accurate, reproducible detection of mutations and copy number variations (CNV) from small amounts of DNA, including single cells, is key for genetic analysis of clinical samples to assist in identifying the best treatment regimen and molecular diagnoses of diseases such as cancer.

Method: To allow accurate detection of both single nucleotide variants (SNVs) and CNVs, we release an enhanced version of our PicoPLEX chemistry that improves sequencing coverage, uniformity, and accuracy while increasing the resolution for CNV detection and retaining reproducibility. This enhanced chemistry named SMARTer PicoPLEX Gold Single Cell DNA-Seq Kit (PicoPLEX Gold) is a single-cell library-prep kit with a simple, four-step protocol to convert single cells into NGS libraries in under three hours with minimum hands-on-time. Libraries prepared from single GM12878 cells using the PicoPLEX Gold kit were sequenced on an Illumina® NextSeq® platform to a depth of ~35 million read pairs (2x150 cycles).

Results: PicoPLEX Gold kit generates >50% genome coverage. This coverage represents a 2-fold improvement over the original PicoPLEX kit, along with a 4X reduction in duplication rates. The kit detected 3.5X more SNVs compared to Multiple Displacement Amplification (MDA) with the same number of reads. PicoPLEX Gold kit produced up to 50% lower allele dropin (false-positive) rates than MDA. The increased coverage and low bias translated to extremely low allele-dropout rates (ADO), ~5X lower than MDA.

Conclusion: A single PicoPLEX Gold kit library enables reliable, high-resolution CNV analysis with shallow sequencing, and an accurate and reproducible SNV and CNV analysis with deeper sequencing.

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P12.140A

Pediatric Systemic Lupus Erythematosus as a manifestation of Constitutional mismatch repair deficiency

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Introduction: Biallelic mutations in any of the four mismatch repair genes *MSH2, MSH6*, *MLH1*, and *PMS2* result in one of the most aggressive childhood cancer predisposition syndromes, termed constitutional mismatch repair deficiency syndrome (CMMRD). In addition to a very high tumor risk, the CMMRD phenotype is often characterized by the presence of signs reminiscent of neurofibromatosis type 1. Pediatric Systemic Lupus Erythematosus (pSLE) is very rare. It has been reported so far in three CMMRD patients and has not been considered a diagnostic feature of the syndrome.

Methods: Two female patients from two different families diagnosed clinically with pSLE presented with features suggestive of CMMRD and were found to have biallelic pathogenic mutations in *MSH6*.

Results: We report two CMMRD female patients diagnosed with pSLE and compare them to the three reported cases. Hence, there are a total of five out of approximately 200 (2.5%) currently reported CMMRD patients that also have pSLE.

Conclusions: Given the rarity of both CMMRD and pSLE this phenotype is significant and should be further explored. pSLE should raise the possible diagnosis of CMMRD if supported by additional indicative features.

H. Toledano: None. **E. Sofrin:** None. **N. Orenstein:** None. **N. Rurman Shahar:** None. **G. Amarilyo:** None. **L. Basel-Salmon:** None. **A. Shuldiner:** A. Employment (full or part-time); Modest; Regeneron. **P. Smirin-Yosef8:** None. **N. Lev El:** None. **M. Aronson:** None. **H. AlTarrah:** None. **K. Wimmer:** None. **U. Tabori:** None. **L. Bazak:** None. **C. Gonzaga-Jauregui:** A. Employment (full or part-time); Modest; Regeneron. **Y. Goldberg:** None.

P12.141B

Impact of Next Generation Sequencing on clinical practice in a Spanish cohort of paediatric and adult patients with solid tumours

D. A. Garcia-Dios[1], C. Perez-Garcia[1], M. J. Garcia-Ruiz[1], R. Palomo[1], C. Ruiz-Lafora[1], C. Lavarino[2], N. gene[2], E. Jantus[3], N. Camarasa[4], J. Forteza[5], C. Camps[3], J. Garcia-Planells[1]

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Survival rates in cancer patients are still improving but at a lower rate than the previous decade. There is a clear need for developing more efficient tests to aid clinicians in choosing the optimum treatment for each patient in a period of time no longer than 10 days. Next-Generation Sequencing (NGS) has been proven to deliver these results in an excellent manner. In this work, we provide the results obtained in a cohort of 75 adult patients suffering from diverse solid tumours as well as 38 paediatric patients. Adult patients obtained a significantly higher benefit from the study. We detected alterations of clinical relevance (SNVs, CNVs, fusion genes and MSI) in more than 90% of the cases. Based on these results, 25% of the patients could benefit from different therapies including FDA-approved drugs or at least one clinical trial. Besides the somatic mutations, 10.7% of the patients harboured a germline pathogenic variant that had not previously detected. Out of the 38 paediatric patients, 15 had the option to join a clinical trial and another three showed a genetic alteration treatable by an FDA-approved therapy. Germline alterations were more common in these patients that reached 28.9% of the cases. Genetic counselling and family studies were offered when relevant. Thanks to the results presented in this work, several patients benefited from an accurate diagnosis that allowed clinicians to approach new therapeutic options. Families of paediatric patients were able to identify germline mutations carried by some of their members that were yet unknown.

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P12.142C

Assessment of a highly-curated somatic oncology mutation database to facilitate identification of clinically important variants in NGS results

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Introduction: The increasing adoption of Next Generation Sequencing (NGS) in molecular profiling of cancer presents a growing need for streamlined interpretation of NGS results in clinical labs.

Methods: We performed an initial assessment of an NGS result interpretation tool called NAVIFY Mutation Profiler (NMP), which enabled us to process a Variant Call Format (VCF) file and generate a report with consensus recommendations of NCCN, ASCO, CAP and ACMG. This annotation tool identifies pathogenic variants and variants of unknown clinical significance (VUS), and groups variants by AMP Tiers. At the time of this assessment, NMP contained curation for ~4,000 variants. In this study, we used NGS results from 38 anonymized clinical cases with known treatment regimens to retrospectively assess NMP as the variant interpretation tool.

Results: NMP correctly associated EGFR TKI therapies options with the corresponding 5 cases. As expected, NMP did not recommend targeted therapies for the 10 chemo-treated control cases. For the subject relapsed against EGFR TKI, NMP correctly interpreted the complex EGFR mutation profile containing both activating (L858R) and drug-resistance (T790M) variants. In addition, out of 22 cases relapsed against ALK TKI crizotinib, NMP correctly marked 14 with crizotinib resistance when a known ALK variant conferring crizotinib resistance was detected. There was limited or no published clinical evidence to interpret the remaining 8 cases of ALK TKI resistance.

Conclusions: NMP correctly interpreted cases containing EGFR and ALK variants in this study. With a highly-curated knowledge base, this tool simplifies NGS clinical reporting by identifying clinically actionable mutations.

M. Ma: A. Employment (full or part-time); Significant; Roche Sequencing Solutions, Inc. **S. Yaung:** A. Employment (full or part-time); Significant; Roche Sequencing Solutions, Inc. **L. Xi:** A. Employment (full or part-time); Significant; Roche Sequencing Solutions, Inc. **C. Ju:** A. Employment (full or part-time); Significant; Roche Sequencing Solutions, Inc. **J. Palma:** A. Employment (full or part-time); Significant; Roche Sequencing Solutions, Inc.

M. Schmid: A. Employment (full or part-time); Significant; Roche Sequencing Solutions, Inc.

P12.143D

The development of SureMASTR HRR assay for SNV, indel and CNV/CNA detection in 17 HRR-related target genes in blood- and FFPE-derived DNA

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Introduction: Growing evidence shows that defects in the homologous recombination repair (HRR) pathway underlie hereditary and sporadic tumorigenesis and that HRR deficiency may dictate the sensitivity of tumors to cancer chemotherapy that rely on defective DNA repair.

Methods: The SureMASTR HRR NGS assay contains 17 selected HRR most relevant genes. These 17 genes were selected based on mutational analysis of a 52 HRR gene panel in 300 high-grade serous ovarian cancer samples, together with the most recent literature research and input from Key Opinion Leaders. The panel is comprised of the full coding region of: *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCA, FANCL, NBN, PALB2, RAD51B, RAD51C, RAD51D, RAD54L* and *TP53*.

Results: The SureMASTR HRR workflow was developed as a single tube assay, together with the MASTR Reporter software application, to enable data quality control, detection of SNPs, indels and CNV/CNAs in the coding regions of 17 selected genes, compromising 1164 amplicons, from blood and FFPE derived DNA. The assay is optimized for cost-efficient use of the NGS capacity and shown to obtain (i) excellent target read mapping (>98%), (ii) uniformity of amplification >96% within 0,2X mean coverage and (iii) low primer dimer (2%). Testing was performed on Illumina's MiSeq and NextSeq sequencers.

Conclusions: The SureMASTR HRR assay is a single plex assay covering 17 most relevant HRR pathway genes, significantly reducing the hands-on time, DNA input, required sequencing capacity and, in combination with the MASTR Reporter data analysis, to provide an accurate and precise workflow.

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P12.144A

Gene expression profiling in aggressive digital papillary adenocarcinoma sheds light on the architecture of a rare sweat gland carcinoma

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Background: Sweat gland carcinomas are rare cutaneous adnexal malignancies. Aggressive digital papillary adenocarcinoma (ADPA) represents a very rare sub-entity, thought to arise almost exclusively from sweat glands of the fingers and toes. The aetiology of sweat gland carcinomas and ADPA is largely unknown. ADPAs are most likely driven by somatic mutations. However, somatic mutation patterns are largely unexplored, creating barriers to the development of effective therapeutic approaches to the treatment of ADPA. Objective: To investigate the transcriptome profile of ADPA using a sample of eight formalin-fixed, paraffin embedded (FFPE) tissue samples of ADPA and healthy control tissue.

Methods: Transcriptome profiling was performed using the Affymetrix PrimeView Human Gene Expression Microarray and findings were validated via reverse-transcription of RNA and real-time qPCR.

Results: Transcriptome analyses showed increased tumour expression of 2,266 genes, with significant involvement of cell cycle, ribosomal and crucial cancer pathways. Our results furthermore point to tumour-overexpression of *FGFR2* ($p = 0.001$).

Conclusions: Our results indicate the involvement of crucial oncogenic driver pathways, highlighting cell cycle and ribosomal pathways in the aetiology of ADPA. Suggested tumour-overexpression of *FGFR2* raises the hope that targeting the FGF/FGFR axis might be a promising treatment for ADPA and probably for the overall group of sweat gland carcinomas. Currently transcriptome profiling is performed in a large sample of eccrine porocarcinoma, the most common sub-entity of sweat gland carcinomas. A

combined analysis of the two entities is currently performed to figure out common and specific pathways.

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P12.146
C*TP53* c.847C&gtT (p.Arg283Cys) variant: A low-risk breast cancer variant?

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Introduction: Based on ACMG criteria and ClinVar data, *TP53* c.847C>T (p.Arg283Cys) represents a variant of unknown significance. It has been detected as germline and somatic mutation in different cancers, but also in controls. It is also reported in population databases (gnomAD, ExAC) with a low frequency. Using transactivation activity, it has been classified as partially-deficient allele, associated with mild disease course.

Material and methods: Aiming to clarify the clinical significance of c.847C>T we studied 1061 breast cancer (BC) patients and 1490 controls from the general population. The methodology included multigene panel testing on MiSeq platform and allele-specific PCR.

Results: Compared to the published allele frequencies (0.02%), c.847C>T variant was significantly more common among our BC patients (10/2122 or 0.47%), but also among our controls (10/2980 or 0.34%). The majority of BC patients carrying c.847C>T mutations had ductal, ER+, PR +, HER2- BC. Seven of the 10 BC patients had familial history for cancer (four of them for BC/OC), but none of them met the Li-Fraumeni syndrome criteria. Only one patient had co-inherited pathogenic mutation in other cancer gene (*BRCA2* c.6937+1G>A) and somatic pathogenic *TP53* mutations were identified in two of the four studied breast tumors.

Conclusion: Here, we report the highest frequency of *TP53* c.847C>T variant, detected among population of R. Macedonia. We further suggest that *TP53* c.847C>T might represent a low-risk BC allele. However, large case control studies, particularly in Balkan populations where it is probably more common, are warranted to confirm our findings.

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P12.147D
Balanced and unbalanced translocations in a series of 2,543 patients with chronic lymphocytic leukemia

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Introduction: Chronic lymphocytic leukemia (CLL) is the most common leukemia of adults in Western countries, and has a highly variable clinical course. Clonal chromosomal aberrations can be detected in 40-50% or in more than 80% of cases depending on the mitogenic agents used. The common recurrent abnormalities are del(13q)[50%], trisomy 12 [15%], del(11q)[15-20%], del(6q) and del(17p) [5%] and t/der(14)(q32) [4-20%]. Chromosomal translocations in CLL are uncommon and most of them occurred within complex karyotypes. OBJECTIVE: To report the translocations identified in patients with CLL diagnosed in three different centers: Hospital Clínic de Barcelona, Hospital Trias i Pujol de Badalona Hospital del Mar de Barcelona and Consorcio Hospital General Universitario de Valencia.

Results: Two hundred and thirty-one (9%) translocations were identified in 2,543 CLL patients, 172 (74%) balanced and 59 (26%) unbalanced. All chromosomes were found to be involved in translocations, with the single exception of the Y chromosome. The chromosomes most frequently involved were chromosome 14 (n=35), 17 (n=29), 2 (n=24), 1 (n=21), 3 (n=13), 5 (n=12), 8,18 (n=11) and 10 (n=10). The recurrent translocation most frequently observed was t(14;18)(q32;q21)(n=28). Translocations were found in karyotypes as the unique chromosomal abnormality (27%), associated with another chromosomal abnormality (25%), and as a part of a complex karyotype (47%).

Conclusion: The infrequency of translocations in CLL makes their identification and reporting interesting for the

recognition of the recurrent ones and the genes involved in this neoplasia.

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P12.148A

High prevalence of *BRCA* mutations among Slovenian triple-negative breast cancer patients

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Introduction: Genetic testing of ovarian cancer patients yields a high rate of *BRCA*-positive results in the Slovenian population. Here, we present genetic testing results in our breast cancer (BC) population fulfilling the hereditary breast and ovarian cancer (HBOC) testing criteria with an emphasis on triple negative (TN) cancers.

Methods: We analysed 402 consecutive BC cases tested using a multigene sequencing panel. We evaluated mutation detection rates in BC-associated genes according to oestrogene/progesterone (ER/PR) and human epidermoid growth factor (HER2) receptor status and further subdivided TN (ER-/PR-/HER2-) BC patients according to their age at diagnosis and family history of HBOC-associated disease.

Results: Pathogenic variants (PV) were detected in 81/402 (19.9%) BC patients in 6 different genes (54.3% *BRCA1*, 18.5% *BRCA2*, 11.1% *CHEK2*, 8.6% *ATM*, 6.2% *PALB2* and 1.2% *TP53)*. 17.7% of ER+/PR+/HER2- BC patients carried a PV, 12.0% were *BRCA1/BRCA2*-positive. In the HER2+ group, 14% were PV carriers – only *BRCA2* variants were seen (6%) with no *BRCA1*-positive cases.

37.9% of our 87 TN BC patients were PV carriers. Most (29.9%) were *BRCA1*-positive, followed by *PALB2* (3.4%) and *BRCA2* (2.3%). TN BC patients with a positive family history (59.8%) were more likely to be *BRCA1/BRCA2*-positive (38.5% vs. 22.9%). When stratified according to age at diagnosis, *BRCA1/BRCA2* mutation detection rate was 66.7% between ages 20-30, 45.8% between ages 30-40, 31.0% between ages 40-50 and 18.2% between ages 50-60.

Conclusions: Testing our TN BC patients produces a high diagnostic yield, possibly due to a high frequency of *BRCA1* carriers in the Slovene population.

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P12.149B

NGS panel testing of negative *BRCA1/2* index patients with Triple-Negative Breast Cancer in Cyprus

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Introduction: Triple-Negative breast cancer (TNBC) is an aggressive form of breast cancer (BC), characterized by lack of expression of the estrogen and progesterone receptors, and 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 124 TNBC patients was sequenced using the TruSight Cancer panel (Illumina). We followed the Genome Analysis ToolKit guidelines and all variants were verified by Sanger Sequencing. Rare variants of uncertain significance (VUS) were evaluated using *in-silico* pathogenicity prediction algorithms and variants predicted as deleterious, were selected for further investigation. Various tools were used to predict the effect of VUS on protein structure and stability.

Results: Five mutations in BC susceptibility genes (*PALB2*(4) & *TP53*(1)) and five mutations in genes not proven to be associated with BC (*ERCC2*(1), *ERCC5*(1), *FANCL*(1) and *PRF1*(2)) were found in 10 TNBC patients. In addition, 20 VUS in established and promising BC susceptibility genes were predicted as deleterious, out of which six were identified in more than one sample (*PALB2* (3), *RAD51C*(2), *BRIP1*(3), *CHEK2*(2), *ATM*(2), *PMS2*(3)).

Conclusions: In summary, five deleterious mutations were identified in five Cypriot TNBC patients in established BC susceptibility genes other than the *BRCA* genes (4.03%). Functional studies, case-control association studies and/or co-segregation analyses are needed to evaluate the VUS for potential pathogenicity. **Grant:** EU H2020; 669026; Establishment of the Bioinformatics Chair at the CING (BIORISE)

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P12.150C

Ultra-high multiplexed 20,000-amplicon NGS panel for tumor mutational burden (TMB) analysis using a rapid 4-hour workflow

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Introduction: In the immuno-oncology field, tumor mutational burden (TMB) is gaining significant importance with its correlation to patient response to checkpoint inhibitor chemotherapy. TMB is generally calculated using whole exome sequencing using laborious hybrid-capture methods. However, targeted sequencing provides better coverage of regions of interest at reduced costs. Here we present CleanPlex® technology for TMB analysis, a 4-hour cost-effective target enriched NGS library preparation method. We demonstrate excellent performance metrics using a highly-multiplexed NGS panel that contains ~20,000 amplicons covering 355 genes for TMB assessment.

Methods: Input of 20 ng of genomic DNA was used to generate CleanPlex sequencing-ready libraries in a 3-step workflow combining target enrichment and library preparation. The protocol includes an ultra-high multiplex PCR step to amplify ~20,000 target regions of interest, a background cleaning step to remove non-specific PCR products, and a final PCR to add Illumina® sequencing adapters and sample indexes. Libraries were sequenced on Illumina NextSeq® platform. Sequencing metrics like on-target rates were calculated, and variants were identified using Paragon Genomics' variant calling algorithm.

Results: Using CleanPlex technology, this prototype TMB panel exhibits >95% uniformity at 0.2X mean, limited GC bias, and >94% detection rate for mutants with 5% allele frequencies. CleanPlex background cleaning step is essential and removes undesirable PCR by-products.

Conclusion: CleanPlex technology is an ultra-high multiplexed PCR-based technology that generates consistent, high quality amplicon libraries with high uniformity, low GC bias, and sensitive variant calling even with ~20,000 amplicons and with a workflow under 4 hours and ideal for TMB analysis.

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P12.151D

Influence of the tumorsuppressor gene ITIH5 on different cervical carcinoma cell-specific 3D tumorspheroid models

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Introduction: Progression from human papillomavirus-induced premalignant cervical intra-epithelial neoplasia to cervical cancer is driven by genetic and epigenetic events. Previous studies revealed a progressive down-regulation of the gene ITIH5 in the course of cervical carcinogenesis. Functional *in vitro* analyses confirmed a suppressive effect of ITIH5 on relevant mechanisms for cancer progression in conventional two-dimensional cell culture. Aim of the current study is to investigate the influence of ITIH5 (with and without use of cytostatics) on different cervical carcinoma cell-specific 3D-tumorspheroid models.

Materials and Methods: ITIH5 was ectopically overexpressed in SiHa and HeLa cells using retroviral transduction. Cell line specific multicellular tumor spheroids (MCTS) are generated using the hanging-drop method. Proliferation-, migration-, invasion- as well as live/dead assays and immunohistochemical analyses are performed to

investigate the influence of ITIH5 on MCTS formation before and after treatment with different cytostatics.

Results: Functionally, ITIH5 overexpression significantly suppressed tumor spheroid growth and spheroid invasiveness in both, SiHa and HeLa spheroids. Immunohistochemical analyses revealed a significant reduction in cell proliferation and hypoxia as well as an increase in apoptosis upon ITIH5 overexpression. So far, an altered susceptibility on cervical MCTS to routinely used cytostatic drug treatment could be not observed. Investigations with novel cytotoxic metal complexes are also ongoing.

Conclusions: Our results provide further evidence of ITIH5 possessing tumor suppressive properties in cervical carcinogenesis. Possible effects of ITIH5 itself and on chemotherapy of cervical carcinoma cell lines using 3D tumorspheroid models may provide a new approach for individualised therapy of cervical cancer in the future.

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P12.152A

FGFR3 and/or CDKN2A/2B somatic mutations are present with high frequency in patients with early stage urothelial cancer

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Urothelial cancer is one of the most common type of cancer worldwide and the molecular defects associated with the initiation of this tumor has great clinical importance. Several studies have implicated the involvement of FGFR3 and CDKN2A/2B genes in urothelial cancerogenesis. We evaluated the frequency of the most common mutations in the FGFR3 gene and deletions of the CDKN2A/2B genes in snap-frozen tumors from 196 patients (163 males, 33 females) with early stage bladder cancers collected immediately after surgery. The methodology included SNaPshot analysis for nine variants in FGFR3 gene (S249C, Y375C, R248C, G372C, A393C, K652E/Q and K652M/T) and MLPA analysis for determination of deletions in CDKN2A/2B genes. A total of 65.2% of patients had somatic defects in one of the two analyzed genes of which 18.5% had only a deletion of CDKN2A/2B genes, 24.1% had a mutation in the FGFR3 gene and 22.6% had a combination of both defects. The most common mutation was S249C (32.1%) followed by Y375C (6.5%). No significant difference was found in the distribution of these defects between men and women, whereas a higher frequency of defects were detected in patients >50 (64.6) compared to patients <50 years of age (28.5) ($p<0.05$). These defects were found with higher frequency in patients with early stage (77.3%) compared to patients with more advanced disease (30.0%) ($p<0.05$). Our results indicate that molecular defects in FGFR3 and/or CDKN2A/2B are important factors in the initiation of the urothelial cancerogeneis in patients with bladder cancer.

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P12.153B

Population-based analysis of germline variations in the *BAP1* gene in patients with uveal melanoma

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Pathogenic germline variants in BRCA1-associated protein 1 (*BAP1*) gene cause BAP1 tumor predisposition syndrome (BAP1-TPDS) with increased risk of several cancers, especially uveal melanoma (UM). Pathogenicity of loss-of-function *BAP1* variants is clear, as opposed to missense and regulatory region variants. We sequenced coding, promoter, UTR, and intronic regions of *BAP1* and analyzed copy number variations in a nation-wide study, enrolling 432 of 533 consecutive Finnish UM patients diagnosed from 2010 to 2017, and one Finnish-Swedish UM family. We analyzed BAP1 functions necessary for tumor suppression using nuclear localization and deubiquitinating activity assays. We found twenty rare variants, and classified five of them as likely pathogenic. Two LOF variants (c.67+1G>T; c.1780_1781insT) are putative founder mutations, and both abolished nuclear localization *in vitro*. The former as well as two heterozygous exon 5 (c.281A>G) and 9 (c.680G>A) missense variants reduced deubiquitinating activity. A deep intronic 25bp deletion in intron 1 caused aberrant splicing *in vitro*. Based on functional studies and family cancer history, three exon 13 missense variants were classified as benign. No copy number variations were found. Frequency

of pathogenic variants was 1.9% (95% confidence interval, 0.8-3.6) overall and 25% among 16 UM families. To establish the pathogenicity of *BAP1* variants, family cancer history and functional assays are essential. Pathogenic variants outside *BAP1* coding region can cause BAP1-TPDS.

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P12.154C
Expression of Wnt signalling pathway in colorectal and breast cancer: Clinicopathological associations

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Introduction: Wnt pathway regulates important cell functions such as proliferation and migration and is dysregulated in colorectal and breast cancer. Thus, it is considered an attractive therapeutic target with many drugs being investigated in clinical trials.

Materials and Methods: The relative mRNA expression levels of Wnt3 ligand, Frizzled7 receptor, and β-catenin in 102 samples of colon cancer and 88 samples of breast cancer were determined by RT-PCR and the ΔΔCt method.

Results: Regarding colorectal carcinomas, increased relative mRNA expression levels of Wnt3 and Frizzled7 were found in 60% and 53% whereas decreased mRNA expression levels of β-catenin were observed in 57% of the cases respectively. Statistically significant linear correlation was observed between the expression of Wnt3 or Frizzled7 and β-catenin. In addition, statistical significant correlation was found between mRNA expression levels of β-catenin and metastasis in lymph nodes. As far as breast carcinomas are concerned, increased relative mRNA expression levels of Wnt3 and β-catenin were found in 49% and 24% of carcinomas, respectively. Interestingly, decreased relative mRNA expression levels were observed in Frizzled7 in 84% of the cases. Expression levels of β-catenin were significantly correlated with patients' age and tumour grade, whereas expression levels of Wnt3 were significantly correlated with tumour grade and expression levels of β-catenin.

Conclusions: The study of Wnt pathway components is of potential clinical importance considering the emergence of prognostic and predictive biomarkers for the administration of targeted therapies against Wnt pathway ligands and receptors, which are used in ongoing clinical trials.

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P12.155D
Mutational landscape of the nucleotide excision repair (NER) deficiency revealed from skin tumours of the Xeroderma pigmentosum subgroups

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Rare autosomal disease Xeroderma Pigmentosum (XP) is characterised by 1000 times increased risk of skin cancer. 8 XP subgroups can be classified as deficient in only global NER (E, C); in both global and transcription coupled NER (A, B, D, G, F) and in translesion synthesis (V). We characterised genomic mutational landscapes of skin tumours (n=10) in XPC, XPD and XPV subgroups representing different types of deficiencies and compared them with tumour type matched sporadic cancers in order to reveal their unique mutagenic properties. XPC samples were characterised by high mutation load (80 mut/Mb) with 3.5 times more di-nucleotide mutations (CC>TT 15%) than in normal samples and very strong transcriptional bias in

intensively transcribed genes (1:5.5 vs. 1:1.5). Excess in the mutation load in XPC in late replication was lower than in sporadic cancers (1.5 times, vs. 2.5 times). XPD tumour demonstrated unique signature of transcription coupled damage on actively transcribed genes and absence of mutation enrichment in the late replicating regions. XPV tumors were characterised by unique mutational signature with excess of CpCpN>CpTpN mutations and depletion of mutations in late replicating regions. NpCpG context was particularly mutable in XPV and XPD skin tumours. Moreover striking unique mutational properties of XP subgroups in the context of nucleosomes and replication fork polarity were observed. Our results reveal unique mutagenic properties of major NER elements which can be mechanistically associated with the diverse syndrome manifestations in XP patients and shed light on our understanding of repair of UV damage in humans.

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P13
Basic mechanisms in molecular and cytogenetics

P13.01A
Maternal mosaicism involving different 18p (micro-) deletions transmitted as a typical 18p deletion to a patient with De Grouchy syndrome I

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Complete loss of the short arm of chromosome 18 ($18p^-$ deletion syndrome or DeGrouchy syndrome I) causes cognitive impairment, ptosis, structural brain malformations, and other features. We describe a family in which the mother of a child with $18p^-$ syndrome showed several different mosaic 18p deletions. Index patient was a 10-year-old girl with intellectual disability (IQ 60), ptosis, white matter abnormalities and mild facial dysmorphism. Her mother and two older brothers were healthy but had mild learning disability.

SNP array in the girl showed near-total loss of 18p (14,7 MB deletion). Chromosome analysis of the mother indicated a normal karyotype in 20/30 metaphases, with 18p deletion in 10/30 metaphases (33%). Interphase FISH with probes for the subtelomeric (D18S552, Vysis) and a more proximal region (RP11-620N7, Empire Genomics, maps to 18p11.23) revealed a more complex mosaic with two distinct cell lines: ~70% of cells carried a microdeletion of the 18p subtelomeric region, and ~30% the large $18p^-$ deletion (involving 18p11.23). In oral buccosa of the mother the ratio was 95% microdeletion and 5% larger deletion. The karyotypes including FISH of the father and one brother were normal. SNP array analysis in the mother and FISH analysis in the second brother are currently underway. While deletions involving chromosome 18 are common, mosaicism for structural rearrangements is exceedingly rare. This is the first report of mosaicism for two different size deletions of chromosome 18p with vertical transmission of the larger deletion to a symptomatic daughter.

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P13.02B
Extrapineal melatonin synthesis during aging

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Diminished expression of many antioxidant factors during aging could be responsible for developing of many diseases associated with aging. One of the most important scavenger of ROS, with more efficient properties than classical antioxidant enzymes, is melatonin. The goal of our study was to better understand the role of melatonin in the antioxidant defense during aging in extrapineal organs.

Since two key enzymes are involved in melatonin synthesis, AA-NAT and ASMT, their gene expression was evaluated in the brain, liver, kidney, heart, skin, and intestine, of 2.5 and 36-months-old Wistar rats. Also, as melatonin might influence the expression of antioxidant enzymes, the activity of SOD, CAT, and GSH in liver was estimated. In addition, microelements (Cu, Zn,and Mn) were also measured in liver due to their role in regulating the activity of antioxidant enzymes.

The finding of significantly increased expression of AA-NAT and HIOMT in liver and skin of older animals suggests that all organs do not age at the same rate. Moreover, the maintaining of antioxidant defense in liver during aging was confirmed by our results that the activity of CAT enzyme was significantly higher in older ones, SOD activity was decreased, while in both age groups there were no differences in GSH activity. Also, a less amount of microelements in liver leads to decreased activity of SOD and increased activity of CAT in older animals.

In all, metabolic products of melatonin, the antioxidant enzymes and microelements in extrapineal organs have an important role in the aging process.

B. Popovic: None.

P13.03C
Transcriptomic analysis of liver organoids reveals altered metabolic pathways in alpha 1 antitrypsin deficiency

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Introduction: Alpha-1 antitrypsin deficiency (AATD) is an inherited disorder associated with lung and liver disease. The most common deficiency allele is the Z allele (Glu342Lys), which causes AAT polymerization and accumulation within the hepatocytes, and predisposes to neonatal hepatitis, hepatic cirrhosis and hepatocellular carcinoma. In addition, the reduction of circulating AAT leads to lung tissue damage. Our objective was to study hepatic disease in organoids from patients with AATD using RNA sequencing technology.

Materials and Methods: We have established liver organoids from AATD patients and controls. Organoids are 3D culture systems in which adult stem cells differentiate to specialized cells under the appropriate culture conditions. We have performed RNA sequencing of liver organoids from controls and patients with ZZ genotype with TruSeq™ Stranded mRNA (Illumina) using a NextSeq System de Illumina. We have determined differentially expressed genes between control and ZZ organoids and identified the pathways enriched for these genes.

Results: The transcriptomic analysis revealed 157 differentially expressed genes in ZZ organoids versus control. Interestingly, a number of genes altered in ZZ organoids are involved in glycan biosynthesis and metabolism, specifically glucosaminoglycans and glucoesphingolipids.

Conclusions: We have identified new molecular targets and pathways involved in AATD hepatic disease. Our results demonstrate that organoids are appropriate systems for modeling hepatic disease in AATD.

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P13.04D
Whole-exome sequencing identifies heterozygous *de novo* stop-loss mutations in HBB resulting in an elongated β-globin chain in two children with severe anemia

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Background: β-Thalassemia is a genetic disease characterized by reduced or absence of β-globin gene (HBB) expression. Some mutations in HBB may produce unstable, abnormal hemoglobins causing (haemolytic) anemia or dominantly inherited β -thalassemia.

Methods: Two unrelated children presented with macrocephaly and severe congenital anemia. They required stem cell transplantations at ages 2 and 4 without clear diagnosis. Since the parents of both probands had no family history of anemia or abnormal Hb, thalassemia was unsuspected. As genetic testing for variants in genes associated with congenital anemia did not result in an explanation for the phenotype, whole-exome sequencing was performed.

Results: We identified two novel, de novo missense stop-loss mutations in HBB (c.422T>C and c.422T>A). The mutations identified caused the loss of a stop codon and an elongation of the translated β-globin chain of 21 amino acids (167 a.a. instead of the usual 146)

due to a new stop codon in the 3' untranslated region (3'UTR) of the HBB gene. The predicted β-globin chains were p.(*148Glnext*21) or p.(*148Lysext*21); the latter variant was called HbMokum.

Discussion: Using whole-exome sequencing, we identified a de novo mutation in two previously undiagnosed children with severe anemia. Both children carry an almost identical heterozygous stop-loss mutation in HBB resulting in an elongated β-globin chain, which most likely produces an unstable hemoglobin. Frameshift mutations in the HBB gene resulting in elongated β-globin chain have been described before, but resulted in shorter β-chains (max. 157 a.a.) and milder phenotypes than the mutations described here.

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P13.06B

Characterization of genomic alterations of cytarabine-resistant AML cells with FLT3-ITD mutation

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Introduction: Cytarabine (ara-C) is a key agent for standard treatment of acute myeloid leukemia (AML). Despite its clinical efficacy, chemoresistance to cytarabine is still a common and serious problem in AML therapy. The presence of an internal tandem duplication (ITD) within the FLT3 gene confers a worse prognosis in patients with AML; however, its underlying mechanisms remain unclear. An ara-C resistant cell line with FLT3-ITD mutation (MV4-11-R) had previously been established. In this study, we aimed to characterize genomic changes for ara-C resistance in the MV4-11-R cells.

Materials and Methods: Cell growth rate was accessed using counting or MTT assay. Cytogenetic alterations were studied using traditional G-banding and spectral karyotyping (SKY) analysis. Gene expressions related to ara-C metabolism, such as ENT1 and DCK, were investigated using qRT-PCR.

Results: Firstly, we confirmed that MV4-11 cells contained the homozygous FLT3-ITD mutation as previously reported. The cell growth rate of MV4-11-R showed dramatically increase to 2.5-fold compared with that of native MV4-11 cells at 96 h culture. Cytogenetic analysis revealed that resistant and parent cells had a modal number of 47~49 chromosomes. The most prominent differences within them are gain of many structural aberrations in resistant cells. Interestingly, 15% of resistant cells showed tetraploid karyotypes and acquired additional structural rearrangements.

Conclusions: Our data found that the resistant clone MV4-11-R exhibited stronger proliferative activity and more complex karyotype than its parent cells. These results suggest that acquisition of resistance to ara-C could be accompanied by global genomic changes and possibly involved chromosomal instability.

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P13.07C

Endoreduplication in cultured lymphocytes: genotoxic or promising finding of compound testing

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Introduction: Endoreduplication is a special type of nuclear polyploidization presented by multiple, uniform copies of chromosomes, more common in plants and invertebrates. Phenomenon of endoreduplication is related to homeostasis maintenance and tumorigenesis. Endopolyploid cells are non-reproductive, but they may possess the potential for DNA double-strand break repair and ability to delay apoptosis. We have compared endoreduplications induction in lymphocyte cultures from healthy donors and psoriatic patients treated by the same antiproliferative compound.

Materials and Methods: Chromosome aberrations analysis was conducted on 100 metaphases per each blood sample and treatment. Lymphocytes were cultivated for 72 h, treated with potential antiproliferative compound, controls were set as well. In order to test antigenotoxic potential of selected bioflavonoids, they were added in normal lymphocyte cultures after induction of genotoxic effects by tested antiproliferative compound.

Results: Results have shown that the antiproliferative compound treatment induces endoreduplications in both psoriatic and normal lymphocyte cultures but in different conditions. In lymphocytes from psoriatic patients, endoreduplications occurred after addition of antiproliferative compound, while the same effect was not observed in normal lymphocytes. We recorded that selected bioflavonoids induce endoreduplications in normal lymphocytes after genotoxic insults of tested compound thereby inhibiting damage progress and reducing genotoxicity.

Conclusions: Our findings show that endoreduplication may be result of genotoxic activity of potential antiproliferative compound in psoriatic patients but not in normal lymphocyte cultures where it may be a potential mechanism for cell survival.

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P13.08D

The fine-tuned balance between expression and degradation regulates the cells' fate

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In order to ensure their survivability cells have evolved numerous mechanisms to control their gene expression. These mechanisms have precise time and space temporal regulation. To investigate them our laboratory used a state of art microscopy system which allows long term live cell imaging and a variety of biochemical approaches. In *Saccharomyces cerevisiae* we excreted the key protein (Dia2) which is part of a modular ubiquitin ligase SCF (Skp1/cullin/F box) that plays a crucial role through normal replication and as well as in the S-phase checkpoint activation. Our results show that when this protein is missing the replication control of the cell is dysregulated and thus the genome expression is destabilized. Furthermore, the dia2 deletion strand also exhibited very intriguing phenotype regarding its cell cycle progression and protein expression. We have observed the same phenotype in higher eukaryotes when the destabilization of proteins is blocked via an inhibitor (MG132), which inhibits the proteasome. This shows that these mechanisms are universally conserved. We speculate that the protein which plays the same role in higher eukaryotes is βTrCP. To investigate that we have silenced the gene in Hela Kyoto cell lines and we have preliminary results that this ubiquitin ligase targets numerous proteins and thus regulates the cell cycle and the whole genome expression.

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P13.09A
High-throughput analysis of driver mutations in *FGFR3* by digital PCR

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Introduction: Mutations occurring at increased frequencies in the germline of older men in the *FGFR3* gene were suggested to confer a selective growth advantage to spermatogonial stem cells. These mutations have been associated with several congenital disorders. Many more mutations have been reported in *FGFR3* for cancer, and it is possible that these expand in the male germline with age.

Materials and Methods: To test an age-related expansion of driver mutations, we are screening sperm and testes of different aged donors with two high-throughput techniques: bead emulsion amplification (BEA) and allele-specific PCR. We are focusing on 13 *de novo* mutations occurring within a ~3kb region of the *FGFR3*, identified in sperm DNA by duplex sequencing (DS) in our laboratory.

Results: With our approach, we verified with BEA similar levels of the c.1118A>G mutation in sperm ($\sim 1.65 \times 10^{-5}$) as measured with DS. We observed an increased mutation frequency with donor's age in 118 sperm donors of different ages. Moreover, there is a ~5-fold difference between young (≤30 years) and middle-aged donors (>45 years). Interestingly, analysis of ~30% of one testis from an 80-year-old man did not show any high-frequency clusters previously observed for other mutations in the *FGFR3* (TDII and ACH).

Conclusions: BEA can validate mutations discovered by DS, and more importantly be used for a more detailed and high-throughput screening of age-related expansions of these in the male germline (sperm and testes) to gain further knowledge about the mechanisms in germline mutagenesis and their potential consequences.

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P13.10B
CDH1 intronic *Cis*-Regulatory Elements control *CDH1* gene tissue-specific expression

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Introduction: Hereditary Diffuse Gastric Cancer (HDGC) is caused by germline *CDH1* coding alterations. Despite not having *CDH1* coding mutations, nor in the remaining exome, >50% of HDGC patients present germline mono-allelic *CDH1* expression, which may indicate a role for *CDH1* locus, beyond the coding sequence. We aimed at dissecting the potential role of intronic *Cis*-Regulatory

Elements (iCREs) in an attempt to identify novel causes of HDGC.

Materials and Methods: We performed a bioinformatics analysis based on open chromatin profiles and prioritized iCRE1 and iCRE8 for further studies. iCREs were cloned in LacZ-reporter constructs and integrated into ColA1 *locus* of mouse embryonic stem cells to generate transgenic mice. Empty-vector mice were used as control for ColA1-driven expression. To test tissue-specific β-galactosidase expression, endoderm (stomach, esophagus, duodenum, liver), ectoderm (heart) and mesoderm (skin) tissues were dissected. iCRE8 was sequenced in HDGC probands.

Results: While no obvious β-galactosidase expression was observed for iCRE1 across tissues, in iCRE8 mice embryos, tissue-specific expression was detected in endodermal-derived tissues (stomach, esophagus and duodenum), where E-cadherin exerts a primordial function. iCRE8 sequence overlaps several genomic and epigenomic regulatory features, is mutated in one HDGC proband and is being explored by 4C-seq and ATAC-seq.

Conclusion: iCRE8 is likely a *CDH1 cis*-regulatory region important for *CDH1* expression in the stomach, and may encompass a novel target for *CDH1* deleterious variants in HDGC patients.

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P13.11C

Identification of a possible chimera following routine peripheral blood screening after a trauma in a pregnant woman

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Hereditary persistence of fetal hemoglobin (HPFH) is a benign condition caused by deletions in the beta-globin gene cluster, or by point mutations in the gamma-globin gene promoter sequences. In heterozygous carriers, 2 to 30% of fetal type of hemoglobin (HbF) continues to be expressed after birth. Offspring of a carrier of a deletion type of HPFH has an increased risk for a hemoglobinopathy if the partner is carrier of a mutation in the beta-globin (HBB) gene, in which mutations are relatively frequent in certain populations.

Peripheral complete blood count was performed in a pregnant woman after a bike trauma, including flow cytometry and hemoglobin electrophoresis. This excluded feto-maternal transfusion but showed a HbF fraction of 15%. Additional testing showed a mosaic (delta-beta)0-thalassemia deletion, identifying the HPFH-type 2. This deletion explains the elevated HbF, usually resulting in a HbF fraction of 30%. To confirm the mosaic deletion, which may explain the lower HbF fraction, SNP array was performed. The mosaic deletion was confirmed, but surprisingly the B allele frequency plot showed a genome-wide complex and patchy chromosomal pattern which could be explained by genetic chimerism, or alternatively, by an around 50% contamination of the DNA sample with the fetus or a close relative.

Short tandem repeat (STR) marker analysis was performed and repeated with an independent blood sample. This test confirmed the genetic chimerism in the patient. Since no Y chromosome contribution was seen, this chimeric state is most probably of no further clinical relevance to the patient.

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P13.13A

Gain-of-function mutations in *KCNN3* encoding the small-conductance Ca^{2+}-activated K^+ channel SK3 cause Zimmermann-Laband syndrome

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Zimmermann-Laband syndrome (ZLS) is characterized by coarse facial features with gingival enlargement, intellectual disability (ID), hypertrichosis, and hypoplasia or aplasia of nails and terminal phalanges. *De novo* missense mutations in *KCNH1* encoding the voltage-gated K^+ channel Eag1 have been identified in a proportion of subjects with ZLS. We report *de novo* missense variants in *KCNN3* in three individuals with typical clinical features of ZLS. KCNN3 (SK3/KCa2.3) constitutes one of three members of the small-conductance Ca^{2+}-activated K^+ (SK) channels that are part of a multiprotein complex consisting of the pore-forming channel subunits, the constitutively bound Ca^{2+} sensor calmodulin, protein kinase CK2 and protein phosphatase 2A. CK2 modulates Ca^{2+} sensitivity of the channels by phosphorylating SK-bound calmodulin. Patch-clamp whole-cell recordings of KCNN3 channel-expressing CHO cells demonstrated that disease-associated mutations result in gain-of function of the mutant channels, characterized by increased Ca^{2+} sensitivity leading to faster and more complete activation of KCNN3 mutant channels. Pretreatment of cells with the CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole revealed basal inhibition of wild-type and mutant KCNN3 channels by CK2. Analogous experiments with the KCNN3 p.Val450Leu mutant previously identified in a family with portal hypertension indicated basal constitutive channel activity and thus a different gain-of-function mechanism compared to the ZLS-associated mutant channels. With the report on *de novo KCNK4* mutations in subjects with facial dysmorphism, hypertrichosis, epilepsy, ID, and gingival overgrowth, we propose to combine the phenotypes caused by mutations in *KCNH1*, *KCNK4* and *KCNN3* in a group of neurological potassium channelopathies caused by an increase in K^+ conductance.

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P13.14B

A novel 22q11.2 deletion mediated by LCR22A-G and meta-analysis in a Greek-Cypriot cohort

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Chromosomal region 22q11.2 is susceptible to genomic rearrangements due to low-copy repeat (LCR) clusters. These high homology sequences mediate meiotic non-allelic homologous recombination, resulting in copy number variations (CNVs), the most common being DiGeorge/Velocardiofacial syndrome (MIM# 192430, 188400).

Here we present a novel deletion within 22q11.2, indicating a novel recombination event mediated by LCR A-G. In light of this new finding, we re-investigated the CNVs within 22q11.2 by revisiting array-CGH data from 2700 prenatal and postnatal cases of Greek-Cypriot patients with intellectual disability and multiple congenital anomalies.

The novel LCR22A-G deletion was identified in a fetus with high NT and atrioventricular septal defect and encompassed DiGeorge/Velocardiofacial Syndrome and the 22q11.2 distal deletion syndrome (MIM# 611867). This is the first report of a LCR22A-G deletion revealing the occurrence of a novel recombination event.

The frequencies of CNVs mediated by different LCRs were shown to be in agreement with published literature. A total of 32/2700 individuals (1.2%) carried CNVs (18 deletions and 14 duplication) mediated by LCR22A to H with the most common (43.75%) spanning the proximal LCR22A-D (14/32). In 15.6% (4/32) and 6.25% (2/32) the CNVs were mediated by the central LCR22B-D and LCR22C-D respectively. The remaining were classified in the distal group with one D-F (3.12%), three F-H (9.4%), two F-G (6.25%), one G-H (3.12%) and one D-G (3.12%).

Newly discovered CNVs within 22q11.2 region may help elucidate pathological genetic mechanisms leading to abnormal phenotypes, thus contributing to better understanding, management and prognosis for 22q11.2 CNV carriers.

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P13.15C
Mosaic ring chromosome 13 syndrome: approach to conventional and molecular cytogenetics

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Introduction: Ring chromosome 13 is a rare chromosome abnormality in which the ends of chromosome 13 join together to form a ring shape. When a ring forms, there may be missing genes where the chromosome's ends fuse together. The aim of this study was to investigate the formation of ring 13 chromosomes by cytogenetic and molecular techniques.

Methods: Standard conventional cytogenetic method with 72 hour-culture was done to peripheral blood lymphocytes. Molecular analysis was performed with subtelomeric FISH and SNP array.

Results: A 5-month-old baby girl, was borned 2530 gr weight, 36-week, from a couple's second IVF pregnancy was sent to us with suspicion of chromosomal anomaly. Her parents were the third generation consanguineous. On physical examination, umbilical hernia, VSD, plump cheeks, depressed nasal bridge, small mouth-thin lip, hypoplastic earlobe and tragus, normal genital development were found. According to ISCN 2016, in 30 metaphases with G-band; 46,XX karyotypes in 3 metaphases; 46,XX,r (13) chromosomes in the other metaphases were found. Normal constitutional karyotypes (46,XY and 46,XX) were detected in her parents. After G-banding, FISH study with 100 nucleus / 20 metaphase and locus-specific subtelomeric probes showed no deletion in ring chromosomes. The SNP array (illumina 656K) was performed to verify the FISH method, no deletion or duplication was detected. Discussion:A few de novo ring 13 chromosome familial cases have been reported in the literature. This is a rare case of individuals with complete-ring 13 chromosome who have normal phenotypes and no loss of genetic material.

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P13.16D
Investigating the potential role of MPHOSPH6 in human telomere length regulation

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Telomere length is associated with atherosclerotic cardiovascular disease and cancer, both of which are leading causes of mortality. Recent studies identified genetic variants in seven loci that associate with human leukocyte telomere length (LTL). Many contain genes known to regulate telomere length, e.g. components of the telomere capping SHELTERIN complex or of the telomerase enzyme required for telomere length maintenance. However, some loci contain no candidate, suggesting they may contain genes with previously unidentified roles in telomere biology.

Our most recent analysis (unpublished) identified a region containing MPHOSPH6 that associates with LTL at a level of genome-wide significance ($P<5x10^{-8}$). Bioinformatics analysis revealed that the LTL associated variants also strongly associate with MPHOSPH6 expression changes, suggesting a link between MPHOSPH6 expression and LTL. MPHOSPH6 is an RNA exosome component and is involved in the maturation of snoRNAs in yeast, therefore we hypothesised MPHOSPH6 could affect LTL through regulating telomerase RNA (TERC) maturation and consequently telomerase activity.

We reduced MPHOSPH6 expression via siRNA-mediated knockdown in HCT116 cells and investigated TERC expression using RT-qPCR and telomerase activity using the telomerase repeated amplification protocol assay.

Significant reductions of MPHOSPH6 expression (4.61-fold decrease, $p<0.01$, $n=3$) were achieved independently with two siRNAs. This resulted in substantial loss of telomerase activity (4.37-fold decrease, $p<0.01$, $n=3$) and reduction of TERC RNA levels (2.06-fold decrease, $p<0.01$, $n=3$).

These data indicate a role for MPHOSPH6 in TERC processing and telomerase activity.

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P13.17A
Molecular weight of a protein-DNA complex can be inferred from native gel electrophoresis

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Introduction: PRDM9 is a multi-domain protein responsible to determine the locations of meiotic recombination

hotspots by recognizing specific DNA motifs via its repetitive array of zinc fingers (ZnFs). Recently it was shown that PRDM9 forms an active multimer; however, neither the size nor the factors inducing the multimerization are known.

Materials and Methods: We performed *in vitro* binding studies, based on gel shift assays, to infer the size of the protein multimer using DNA fragments that increased in length, containing either one or two specific target sites. We tested different protein constructs including truncated protein versions, and analyzed the molecular weight and the corresponding migration distance of binding complexes in relationship to known standards to further determine the protein stoichiometry.

Results: We observed that PRDM9 multimerizes to an active trimer which is formed within the repetitive DNA-binding ZnF domain and 5 out of 11 ZnF repeats are sufficient to form the trimer. Moreover, we demonstrated that only one of the ZnF arrays within the trimer contacts the DNA, whereas the remaining two ZnFs likely perform ZnF-ZnF interaction to maintain the multimeric conformation.

Conclusions: In this work, we report a method using native gel electrophoresis to infer the size, as well as, to investigate the molecular interaction of a multimeric protein with DNA. This simple method is suitable especially for proteins with complex structures or repetitive motifs as it is the case for many ZnF proteins.

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P13.19C

Nicking of double-stranded nucleosomal cell free DNA in sepsis patients

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Introduction: Elevated levels of cell free DNA (cfDNA) in plasma are associated with various medical emergencies such as sepsis and stroke and trauma. In sepsis, levels of cfDNA correlate with disease severity. The goal of this study was to examine if structural damage in cfDNA is present in sepsis patients.

Materials and Methods: Samples were collected from nine consenting patients (age 18+) admitted with severe sepsis to the two ICU´s of Landspitali from December 2016 until May 2017. Samples were also collected from five healthy controls. The sample DNA was gently isolated using the Genomic Mini AX kit according to protocol. Northern Lights assay (NLA) was used to detect structural damage in cfDNA such as single/double-stranded breaks, bends, and inter/intra strand links. Study was approved by National Bioethics Committee and Icelandic Data Authority.

Results: Damage in cfDNA was observed in all the sepsis patients including: Nicking in double-stranded nucleosomal fragments, incomplete degradation of nucleosomal DNA, and lesions causing bending of DNA molecules. Elevated levels of plasma cfDNA were lowered after treatment and cfDNA damage decreased.

Conclusion: To our knowledge this is the first demonstration of cfDNA damage in sepsis patients. Damage in cfDNA might reflect increased damage to DNA or lack of repair associated with cell death prior to release into the bloodstream. Alternatively, these results might reflect damage to nucleosomal DNA in plasma. Damage in cfDNA might contribute to intense immune stimulation in sepsis patients and be a biomarker for sepsis. Icelandic Technology Development Fund 142709-0613

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P13.20D

Small supernumerary marker chromosomes as a reason for infertility can only be reliably assessed by cytogenetics & molecular cytogenetics

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Impaired spermatogenesis is indicative for gross chromosomal anomalies, including small supernumerary marker chromosomes (sSMCs). sSMCs can easily, reliable and for

due to their specific features like mosaicism and often pure heterochromatic constitution be exclusively identified by a standard chromosome analyses. Almost 200 cases were included in this study, which could show that sSMC can be optimally characterized by single cell directed (molecular) cytogenetics. In infertile males sSMC derive predominantly from one of the acrocentric chromosomes, especially #15, #14 and #22. Altered spermiograms were found in 62% of these male sSMC-carriers, while the remainder ones had infertility in connection with repeated abortions in partnership. Meta analyses for detectability of sSMC by array-comparative genomic hybridization revealed that 81-87% of the cases would not have been picked up by exclusive use of this approach. As impaired spermatogenesis is known to be indicative for gross chromosomal anomalies in infertile male patients, it is obvious from this study that sSMC presence also needs to be considered. However, sSMC can only be reliably detected by standard karyotyping and not by modern high throughput approaches, as proven here.

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P13.21A

The Fanconi anemia proteins FANCM and FAAP24 limit telomere instability and BLM/Polδ-dependent break induced replication in the alternative lengthening of telomeres (ALT) pathway

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~15% of human tumours use the Alternative Lengthening of Telomere (ALT)mechanism to maintain telomeres. We previously demonstrated that depletion of FANCD2, a member of the BRCA/Fanconi anaemia DNA repair pathway, causes a hyper-ALT phenotype. We now extend our analysis to the DNA translocase FANCM and its partner FAAP24.

We find siRNA depletion of FANCM or FAAP24 in ALT human cells results in increased replicative stress at telomeres, rapid telomere elongation, and 4-5 fold increases in ECTR DNA number. Importantly, FANCD2 localizes to telomeric foci in FANCM or FAAP24 depleted ALT cells, suggesting that FANCM and FAAP24 protect against telomeric replicative stress independently of other Fanconi anaemia proteins.

siRNA depletion of the BLM DNA helicase or the polδ DNA polymerase in ALT cells resulted in telomere shortening and markedly fewer ECTR DNA molecules. Depletion also suppressed the excessive ECTR production and increased telomere length seen in FANCM and FAAP24-deficient ALT cells, suggesting that these abnormalities are an exaggeration of the ALT phenotype.

Our results support models in which failed telomeric replication forks give rise to DNA breaks and gaps used by BLM/polδ-dependent break induced replication to drive ALT and produce ECTR DNA. FANCM and FAAP24 restrain ALT by preventing collapse of stalled telomeric replication forks. Consequently, loss of FANCM or FAAP24 increases the natural substrate for ALT, failed telomeric replication forks, which results in a hyper-ALT phenotype. As the hyper-ALT phenotype is associated with cell death, disruption of the BRCA/Fanconi anemia pathway may be a novel treatment strategy for ALT tumours.

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P13.22B

A Molecular diagnosis of WOREE syndrome that required genome sequencing to identify the second hit pathogenic variant in *WWOX*

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Introduction: Bi-allelic germline variants in *WWOX* (WW domain-containing oxidoreductase) have been implicated in spinocerebellar ataxia (SCA) and WOREE syndrome (WWOX-related epileptic encephalopathy). We report here a molecular diagnosis of WOREE syndrome that required genome sequencing (GS) to identify the second hit.

Material and Methods: Solo exome sequencing (ES) followed by trio GS were performed on a girl presenting with generalized hypotonia, myoclonic epilepsy, ataxia, psychomotor delay, stereotypies, strabismus and cerebral atrophy.

Results: After ES, two compound heterozygous missense mutations in SLC30A10 were considered as candidate variations for the disease in this patient and classified of unknown significance. A missense variation (p.Thr12Arg) in *WWOX* without any other variation in a gene associated with an autosomal recessive encephalopathy did not

particularly catch our attention at first during analysis of ES data. The identification of a second hit was only possible after GS which revealed a complex CNV consisting of two intronic deletions in introns 4 and 5 associated with an inversion of the fifth exon of WWOX. The *deletions/inversion* was confirmed at the genomic level for both intronic junction fragments. RNA studies showed that this intragenic rearrangement resulted in abnormal pre-mRNA splicing.

Conclusions: GS allowed us to identify the second hit pathogenic variant in a patient heterozygous for a missense variant after ES. This compound heterozygous genotype is likely responsible for the severe epileptic encephalopathy in this patient, a phenotype consistent with a WOREE syndrome. Interestingly, this patient is the first one presenting a WWOX-related phenotype associating epileptic encephalopathy and ataxia.

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P13.23C

Balanced X-autosome translocations and premature ovarian failure are associated with altered expression of growth factors, junction organization and immune pathways

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Patients with balanced X-autosome translocations and premature ovarian failure (POF) are an interesting paradigm to study positioning effect of chromosome segments. They present breakpoints that do not disrupt genes related to the phenotype and map within cytobands Xq13-Xq21, from which 80% cluster in Xq21. As deletions within Xq21 do not cause POF, and since different breakpoints and translocation with different autosomes lead to the same phenotype, we hypothesized a "position effect" as possible mechanism. We fine-mapped the breakpoints in six patients with POF and balanced Xq-autosome translocations, established lymphoblastoid cell lines from patients and matched female controls and profiled their transcriptome through RNA-seq. The 68 genes differentially expressed in the patients' group are enriched for genes encoding important proteins for the organization of cell junctions and the immune response. Previous transcriptome studies pinpointed that cellular assembly and maintenance are among the most expressed pathways in ovarian cells. Additionally, GWAS showed that immune pathway-related genes were associated with age at normal menopause, allowing to postulate that genes involved in this regulation could also be involved in POF. As the perturbed transcripts are neither mapping to the X chromosome, nor to the autosome breakpoint, this might indicate that the effect is indirect, and the phenotype is triggered by perturbations of normal contacts between genes and their regulatory elements. To further challenge this model, we are currently assessing the chromatin accessibility of the same cell lines. These results should help elucidating the impact of derivative chromosomes repositioning within interphase nuclei. Financial support: FAPESP#2017/20847-9.

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P14

New diagnostic approaches - Technical aspects - Quality control

P14.001D

Specific genotype variants have distinct 3D facial gestalt morphometry: pilot study on Czech cases with 16p11.2 microdeletions / microduplications and *STAT3* variants

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Craniofacial assessment is instrumental in the diagnostics of syndromic disorders, where typical facial features lead to etiological diagnosis. However, massively parallel sequencing brought the commonly applied "genotype first" diagnostic strategy. In such cases 3D facial morphometry (3D) digitalizes patients' facial phenotype and enables phenotype driven variant prioritization.

Here we report 2 groups of syndromic cases versus age and sex-matched population controls. Firstly, patients with recurrent microdeletions / microduplications in the 16p11.2 proximal region (diagnosed by aCGH, SNP array, FISH and MLPA) who had similar pattern in both CNVs, which were only partially mirrored and were more pronounced in cases

with microdeletions. These 3D observations are in accordance with previously reported differential penetrance of other phenotypic features (e.g. head circumference, BMI), which is in general lower in cases with microduplications in patients with 16p11.2 syndrome. Secondly, we analyzed 2 cases with combined immunodeficiency and pathogenic variants in *STAT3* with one having a loss of function, while the other has a gain of function. Interestingly, reverse 3D phenotyping found almost "inverse" facial features.

Specific patterns of 3D facial phenotype and also a certain degree of mirroring of phenotypes in opposite genotypes have been found in presented groups of patients. Our current results support the use of 3D as a useful tool in analyzing and comparing facial features, including characterization of the overall age-specific facial gestalt in syndromes without consistent facial phenotype and/or in ultrarare genetic disorders where there are limited data on their facial phenotype.

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P14.002A
Identification of disease causing aberrant splice events in lymphocytes

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Introduction: Whole exome sequencing (WES) revolutionized the field of Human Genetics with a quick and accurate method to identify disease causing variants, with diagnostic rates of approximately 25-50%. Whole genome sequencing (WGS) improves the diagnostic rate, but not dramatically, mainly due to an interpretation gap, yet to be closed. RNAseq was suggested as a complementary method, allowing direct observation of the transcriptome and the identification of missplicing events derived from deep intronic or coding sequences variants, that are difficult to interpret, even when identified with WES or WGS. Although RNAseq was proved to improve diagnostic yield, the need to obtain RNA from the relevant disease tissue is an obstacle to implementing it as a clinical method.

Methods: 56 cases of varied phenotypes were tested by PCR for specific gene missplicing or abnormal expression in RNA extracted from lymphocytes. Most cases were identified with WES, where a splice variant was suspected.

Results: 27%(15/56) of tested genes were not expected to be expressed in whole blood according to the GTEx database, but only in 5%(3/56) cases we were not able to detect a transcript. 23 case (41%) validated the disease causing variant, resulted in misspliced transcripts.

Conclusions: The cases validated with RNA extracted from lymphocytes included developmental delay, hypotonia, ataxia and other phenotypes not involving the circulating system, demonstrating the feasibility of detecting aberrant splicing events in an accessible tissue, suggesting that improving the ability to detect rare transcripts with RNAseq, will allow this method to serve as a clinical diagnostic tool.

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P14.003B
Sequencing the previously unsequencable using amplification-free targeted enrichment powered by CRSIPR-Cas9

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Genomic regions with extreme base composition bias and repetitive sequences have long proven challenging for targeted enrichment methods, as they rely upon some form of amplification. Similarly, most DNA sequencing technologies struggle to faithfully sequence regions of low complexity. This has especially been trying for repeat expansion disorders such as Fragile X disease, Huntington disease and various Ataxias, where the repetitive elements range from several hundreds of bases to tens of kilobases. We have developed a robust, amplification-free targeted enrichment technique, called No-Amp Targeted Sequencing, that employs the CRISPR/Cas9 system. In conjunction with SMRT Sequencing, which delivers long reads spanning the entire repeat expansion, high consensus accuracy, and uniform coverage, these previously inaccessible regions are now accessible. This method is completely amplification-free, therefore removing any PCR errors and biases from the experiment. Furthermore, this technique also preserves native DNA molecules, allowing for direct detection and characterization of epigenetic signatures. The No-Amp method is a two-day protocol that is compatible with multiplexing of multiple targets and multiple samples in a single reaction, using as little as 1 μg of genomic DNA input per sample. We have successfully targeted a number of repeat expansion disorder loci (*HTT*,*FMR1*, *ATXN10*, *TCF4*, *C9orf72*) with alleles as long as >2700 repeat unites (>13 kb). Using the No-Amp method we have isolated hundreds of individual on-target molecules, allowing for reliable repeat size estimation, mosaicism detection and

identification of interruption sequences - all aspects of repeat expansion disorders which are important for better understanding the underlying disease mechanisms.

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P14.005D
Finland's largest next generation biobank provides valuable resources for genomics research

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Background: Studying the genetics of complex diseases requires large genotyped datasets of tens of thousands of individuals, while whole-exome (WES) or whole-genome (WGS) sequencing data is often necessary for studying rare variants. High-quality analyses require high-quality phenotype data and well-defined disease endpoints. Since 1992 the Finnish National Institute for Health and Welfare (THL) has systematically collected DNA samples from all participants of its health examination surveys. These samples have now been processed, and vast amounts of genomic data is available to researchers through THL Biobank.

Materials and Methods: THL Biobank collections include genetic data from >10 Finnish study cohorts. Currently the collections include: GWAS chip data N>74,000, of which >50,000 imputed in the FinnGen project to a high-coverage population-specific reference panel (N>100,000 reached within a year); WES N>12,500; WGS N>2,000; methylation data N>500; gene expression data N>550. All study participants can be followed up (for up to 25 years) from national registers for disease endpoints and drug purchases, and the vast majority has wide availability of questionnaire and laboratory variables. Additionally, there are cells available for >10,000, RNA for >3,000 and NMR metabolomics for >40,000 of these.

Results and Conclusions: THL Biobank data is available for research use for researchers and companies worldwide. Since 2015, 98 biobank projects have been initiated, including interesting projects ranging from pharmacogenomics and returning genetic data to study participants to cardiovascular and cancer research. Of the biobank projects 93% (91/98) have included genomic data, thus genetic data is of high interest for researchers seeking biobank material.

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P14.006A
Splinted ligation adapter tagging (SPLAT), a sensitive and versatile library preparation method for single stranded DNA

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Introduction: Next Generation Sequencing (NGS) combined with bisulfite conversion is the gold standard technique for interrogation of DNA methylation levels. However, sample preparation for whole genome bisulfite sequencing (WGBS) is challenging due to the side effects of the bisulfite treatment causing DNA strand breakage and single strandedness. We recently developed Splinted Ligation Adapter Tagging (SPLAT), a sensitive library preparation method for WGBS (Raine et al, 2017, NAR). To evaluate platform-related bias, we have evaluated SPLAT in comparison with commercial WGBS library preparation kits on different Illumina platforms (Raine et al, 2018, PLoS One).

Materials and Methods: We have optimized the SPLAT protocol for preparing sequencing libraries from samples containing low quantities of single-stranded DNA or mixtures of single/double-stranded DNA. This includes samples of ultra low-input (picograms) and direct WGBS library preparation from cells.

Results and Conclusion: We show that SPLAT is a fast simple and cost efficient library preparation method that produces sequencing libraries of high complexity, uniform genome coverage and compares favorably to commercial WGBS kit. Moreover, SPLAT can be applied to low (and

ultra-low) levels of DNA and may be useful for cell-free (liquid biopsy) DNA library preparation.

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P14.007B
BRCA germline mutation detection in cervical exfoliated cells by next-generation sequencing

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Introduction: Female deleterious germline BRCA1 and BRCA2 (BRCA1/2) gene mutation are at increased risk for developing breast or ovarian cancer. [1] The aim of this study was to develop an NGS-based workflow for BRCA mutation screening in cervical exfoliated cells, which are usually used to assay human papillomavirus (HPV) infection in primary cervical cancer screening.

Materials and Methods: Genomic DNA was extracted from 64 cervical swab specimens from healthy women. And an NGS-based workflow was designed using the MGICare BRCA1/2 multiplex PCR amplicon libraries followed by MGISEQ-2000RS sequencer. A reference BRCA1/2 mutation dataset from whole genome sequencing (WGS) of these samples was used to assess detection performance.

Results: Total 680 BRCA1/2 benign variants were found in these swab specimens and the consistency with WGS data was 99.41% (671 in 675 reference BRCA1/2 mutations). The same reference variant (chr13, 32912299, T>C, 50%) from four samples not detected by multiplex PCR-NGS was with low sequencing depth (7x, 12x, 19x, and 19x, respectively) in WGS data and it does not exist confirming by Sanger sequencing. Another 9 real BRCA1/2 variants detected by multiplex PCR-NGS were also verified by Sanger sequencing. No pathogenic mutations were found in these healthy women.

Conclusions: We found better SNP detection accuracy than WGS and new insights into BRCA mutation screening accompanying HPV screening.

Reference:

1. Chan, M., et al. (2012).Development of a next-generation sequencing method for BRCA mutation screening: a comparison between a high-throughput and a benchtop platform. J Mol Diagn 14, 602-612.

M. He: None. **M. Li:** A. Employment (full or part-time); Modest; MGI Tech Co., Ltd. **C. Huang:** A. Employment (full or part-time); Modest; MGI Tech Co., Ltd. **Z. Mei:** A. Employment (full or part-time); Modest; MGI Tech Co., Ltd. **F. Chen:** A. Employment (full or part-time); Modest; MGI Tech Co., Ltd. **H. Jiang:** A. Employment (full or part-time); Modest; MGI Tech Co., Ltd..

P14.008C
Variants with reduced variant fractions in NGS-based germline diagnostics for hereditary breast and ovarian cancer

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Next generation sequencing (NGS) is the standard for detecting germline variants in a routine diagnostic setting. The high sensitivity of NGS allows the detection and accurate quantification of variants showing low variant fractions (VFs). In 11 index patients with familial breast and/or ovarian cancer, NGS-analysis of blood-derived DNA identified 11 distinct deleterious variants (IARC class 4/5) with unusually low VFs of 10-30%. These were found in four established cancer predisposition genes (7x*TP53*, 2x*CHEK2*, 1x*BRCA1* and 1x*MSH2*). Complementary NGS-analysis of DNA derived from tumour or non-diseased tissue (available from 8 of 11 patients) confirmed three variants (2x*TP53*, 1x*BRCA1*). Inheritance of the deleterious mutation to the offspring was seen in the case of the *BRCA1*-positive patient. The five remaining variants (3x*TP53*, 2x*CHEK2*) were absent in the complementary tested tissue and therefore may be blood-specific. One likely scenario for the occurrence of these variants is chemotherapy-induced clonal hematopoiesis, as recently shown for *TP53*. In line with this hypothesis, four of the five patients (3x*TP53*, 1x*CHEK2*) received chemotherapy (carboplatin) prior to blood draw. In summary, variants with low VFs may be due to somatic mosaicism (e.g. clonal hematopoiesis) or mosaics affecting germline. These data indicate that VFs needs to be considered in the interpretation of NGS results and variants with low VFs need to be confirmed in independent tissues. This can provide insights into the cause of the tumor disease and possible heredity of mutations to provide the patient with accurate counseling.

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P14.009D

Estimation of tumor mutational burden based on direct comparison to a panel of normals or a panel of tumors

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The amount of somatic variants in cancer, the tumor mutational burden (TMB) has been associated with positive outcome of immune checkpoint inhibitor therapy in several cancer entities. Distinguishing somatic variants from high levels of germline variants or sequencing artifacts can be accomplished by sequencing a matched normal sample from the same individual, which is often not available in routine diagnosis. While raw variant calls can be filtered for germline variants by population frequency in public databases, sequencing artifacts are specific to wet-lab methodology. We employed standard open source software for alignment (bwa) and variant calling (GATK4 mutect2) combined with a one on one artifact filtering different to GATK's best practices. Variants in in the tumor were directly compared to the alignments of a panel of normals rather than being called in each sample and then combined in a vcf file which then serves as a blacklist. We estimated TMB on a custom gene panel of ~1GB coding exons and compared results of 9 samples to several sequencing panels and software solutions of commercial vendors. Our approach showed different levels of correlation with tested alternative solutions, peaking R^2=0.995 (Illumina TSO500) and R^2=0.959 (QIAseq TMB Panel). We currently evaluate the pipeline in tumor only mode and will present a detailed comparison.

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P14.010A

Low frequency variant detection from highly degraded DNA and RNA

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Clinical oncology samples are often limited in quality and quantity, making NGS-based detection of pathologic variants challenging. Sensitive and accurate detection of low-frequency variants can be accomplished using hybrid capture and ultra-deep targeted sequencing, but rely on high conversion during library construction. Size-selection is required to remove adapter-dimers resulting in significant sample loss and reduced sensitivity to low frequency variants. Specificity also suffers as many low-frequency artifacts arise during sample preparation and hybrid capture. Duplexed molecular barcoding can eliminate these artifacts, but conventional duplexed adapters are difficult to synthesize and purify, leading to reduced ligation efficiency. We present the IDT library preparation kit optimized for low-input and degraded samples. Our chemistry relies on a proprietary engineered DNA ligase and sequencing adapters that prevent chimeras and suppress dimer-formation negating the need for size-selection. We adopted the workflow for both DNA and RNA applications and demonstrated efficacy using diverse sample types. To assess sensitivity, we created libraries using mixtures of genome in a bottle gDNA and performed hybrid capture using a custom panel targeting single nucleotide variants (SNVs), copy number variants (CNVs), and gene fusions. When compared to commercially available methods, our approach yielded a 1.5 to 4-fold increase in library complexity with improved sensitivity to <0.25% variants using 1-25 ng cfDNA, and <0.5% using 25-250 ng FFPE DNA. RNA libraries were constructed from FFPE NGS reference standards to evaluate fusion detection. Our method provides superior sensitivity and specificity for detection of low-frequency variants, even with highly degraded DNA and RNA.

U. Das Chakravarty: A. Employment (full or part-time); Modest; Integrated DNA Technologies, Inc. **A. Royall:** A. Employment (full or part-time); Modest; Integrated DNA Technologies, Inc. **M. Hong:** A. Employment (full or part-time); Modest; Integrated DNA Technologies, Inc. **H. Huang:** A. Employment (full or part-time); Modest; Integrated DNA Technologies, Inc. **K. Lai:** A. Employment (full or part-time); Modest; Integrated DNA Technologies, Inc. **K. Dilger:** A. Employment (full or part-time); Modest; Integrated DNA Technologies, Inc. **K. Bryan:** A. Employment (full or part-time); Modest; Integrated DNA Technologies, Inc. **Y. Wang:** A. Employment (full or part-time); Modest; Integrated DNA Technologies, Inc. **L. Lewis:** A. Employment (full or part-time); Modest; Integrated DNA Technologies, Inc. **S. Rose:** A. Employment (full or part-time); Modest; Integrated DNA Technologies,

Inc. **Y. Zheng:** A. Employment (full or part-time); Modest; Integrated DNA Technologies, Inc..

P14.011B

Allelic drop-out is a common phenomenon reducing the diagnostic yield of PCR-based target sequencing

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Allelic drop-out (ADO) is a known phenomenon of selective allele amplification representing the potential problem of correct DNA diagnostics. Both NGS and Sanger sequencing are PCR-based methods, Sanger sequencing is used to verify NGS results. The purpose of this study is to demonstrate the incidence of ADO reducing the diagnostic yield in primary cardiomyopathy genetic testing via semiconductor NGS and Sanger sequencing of target gene panels.

Methods: We have developed 3 AmpliSeq custom gene panels for mutational screening: "K^+/Na^+ ion channels", "Desmosomal proteins", "Sarcomeric proteins", contains 1049 primer pairs (37 genes) totally, 152 kb. About 140 probands were screened with at least one of these gene panels. AmpliSeq sequences were analyzed in silico and visually compared with Sanger control sequences, noting the facts of heterozygosity loss.

Results: We have detected 12 ADO cases both in Sanger (5 cases) and AmpliSeq (7 cases) sequencing data. All ADO events happened due to frequent or rare SNVs in the oligoprimer annealing sites and were detected due to mismatch in frequent SNPs zygosity nearby. Three pathogenic variants would be missed if were not revealed by re-sequencing with alternative method and alternative oligos.

Conclusion: All PCR-based methods have a risk of ADO leading to a decrease of diagnostic yield of genetic testing. ADO can theoretically affect 1% amplicons. It seems that real scope of ADO might be much higher and depends on numbers of primer pairs. The software for ADO detection is needed. This work was supported by RNF grant №16-15-10421.

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P14.012C

Utilizing long-read amplicon sequencing for comprehensive pan-ethnic spinal muscular atrophy (SMA) carrier screening

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Introduction: Carrier screening for spinal muscular atrophy (SMA) often involves copy number assay of *SMN1* exon 7 dosage. These assays are typically insensitive to 2 *cis* copies of *SMN1* on one chromosome and none on the other; a scenario which accounts for 8%-29% of carrier states, depending on ethnicity. Thus, we established a new pan-ethnic assay to improve *cis* allele carrier detection.

Materials and Methods: 96 Jewish subjects, from various countries of origin across 4 continents were selected for study. An established qPCR assay was performed on all samples to assay *SMN1* exon7 dosage. Subsequently, a 16kb region, encompassing *SMN1*/2 exon 7 and flanking polymorphic SNP sites, was PCR amplified from each sample with barcoded primers for SMRT sequencing on a Sequel System (Pacific Biosciences). Consensus reads were uniquely mapped to either *SMN1* or *SMN2* and c.840C>T status was independently determined. Finally, *SMN1* exon 7 dosage was validated by a commercial MLPA kit (MRC Holland).

Results: The combined qPCR and long-read amplicon analysis correctly identified the allelic state of 95 samples passing quality control. Interestingly, *SMN1>SMN2* gene conversion events were identified in both a zero copy and an unrelated 1 copy carrier. In addition, *SMN2>SMN1* gene conversion events were also detected in 3 *cis* carrier controls and 3 other unrelated 3-copy individuals. None of the gene converted samples were of the same ethnicity.

Conclusion: We describe a novel rapid and low-cost pan-ethnic carrier screen for SMA, expanding the scope of SMA carrier detection beyond that of currently established screening methods.

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P14.013D

The epigenomic landscape of coverage variability in low-pass plasma cell-free DNA sequencing from non-invasive prenatal screening

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Low-pass whole-genome sequencing of plasma cell-free DNA (cfDNA) has been widely adopted as an assay technology for a variety of non-invasive applications, including prenatal screening of fetal genomic abnormalities. These aligned reads can be summarized as binned read counts overlapping contiguous partitions of the genome, providing evidence of copy-number aberrations and aneuploidy. However, our experience indicates cfDNA sequencing exhibits a greater degree of regional coverage variability both within and across samples than encountered in typical whole-genome sequencing. Moreover, we have found that traditional intra-sample GC content and mappability correction are insufficient to fully account for this variability, resulting in temporal trends in chromosomal coverages across samples. To comprehensively explore genomic features that may contribute to this variability, we examined cfDNA sequencing coverage profiles for N = 2258 normal non-invasive prenatal screening (NIPS) assay results. Autosomal GC-corrected bin-level coverage (10kb bin size) was modeled as a function of assay date using smoothing splines, adjusting for estimated fetal fraction. We identified 42,683 10kb segments (16.6%) throughout the genome with significant temporal associations (FDR <0.05). Next, we explored genomic annotation enrichment among the significant regions using >8000 epigenomic features from the Roadmap Epigenomics Project and other public resources. Hypergeometric enrichment testing identified 817 significant associations (Bonferroni-adjusted P<0.05), with the top enrichment results corresponding to H3K9me3 histone modifications in leukocyte cell-lines. These findings suggest cell-specific heterochromatin composition may contribute to inter- and intra-sample coverage correlation patterns via differential cfDNA degradation, which could be leveraged to improve coverage normalization and increase screening assay performance.

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High error rate in a specific region of the *CFTR* gene may lead to false variant findings when using massively parallel sequencing in molecular diagnostics of cystic fibrosis

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Introduction: Cystic fibrosis is a relatively common but severe autosomal recessive disorder. Molecular-genetic testing of the associated *CFTR* gene is routinely performed in standard clinical care. During a validation process of our massively-parallel-sequencing (MPS) results, we identified a region around exon_10 that was found to be particularly prone to specific sequencing errors.

Material and Methods: MPS data were generated using different sequencing panels on Illumina MiSeq/NextSeq platforms. Read mapping and variant calling was performed using Bowtie2 and Vardict, respectively. Genotyping of the TG_n/T_n motif was performed using Dante, an alignment free STR genotyping tool. Exon_10 and surrounding intronic regions were validated by Sanger sequencing.

Results: We identified several types of false variants in this region. One group was found to be caused by a highly homologous but not fully matching region in chromosome 20. Since this region was not present in GRCh37, corresponding reads were attracted by the *CFTR* gene creating thus false variant calls. Using GRCh38 these variants disappeared. The same homologous region caused problems also to our STR genotyping tool which detected an additional TG/T allele. Another group was found to be typically associated to sequencing reads directly spanning the intronic TG_n/T_n polymorphic locus. These were associated to larger repeat numbers and had their origin in repeat-induced sequencing errors.

Conclusion: The mentioned *CTFR* region revealed findings worth of investigations, possibly having implications for another genomic regions too. Such error sources should be kept in mind when evaluating variants and considering additional validation analyses. Supported by: VaV_MZSR_2018/46-SAV-5 and VEGA_1/0433/19.

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P14.015B

Clinical reporting of NGS data: A systematic Nordic collaborative, peer-reviewed benchmarking

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The clinical genomics report details key findings from the interpretation of diagnostic next-generation sequencing (NGS) data and represents a core hand-off between specialized clinical genomics laboratories and the broader healthcare community. However, the design of clinical genetics reports is not standardized, where misunderstanding of results, limitations or key findings can lead to incorrect therapeutic decisions and directly impact patient management. In this study, we first reviewed existing guidelines and regulations on clinical reporting. To evaluate variation across clinical reports, we then conducted a peer-reviewed benchmarking exercise among members of the Nordic Alliance for Clinical Genomics (NACG) from Denmark, Finland, Iceland, Norway and Sweden. Participating laboratories were given three fictional clinical cases, from which they produced clinical reports using their current production pipelines. Participants then evaluated each other's reports using a structured questionnaire. The results of the benchmarking showed a significant variation of reporting on one hand, and difficulties in comprehending key information on the other. Finally, we performed a series of semi-structured interviews with specialists involved in medical genetic testing and end-users of the reports. Through these activities, we identified gaps in existing guidelines for clinical reporting of genetic variants as well as pain points for effective information transfer. The final phase of this project focused on the report end user's needs and challenges as input for a redesign of the clinical genomics report, with the aim to ensure effective and accurate flow of information from the genomic laboratory to the end user.

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P14.016C
Quality improvement in clinical NGS through a peer-driven Nordic collaboration

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Although the technology for high-quality next-generation sequencing (NGS) data is widely available in a research context, clinical implementation of high-throughput NGS-based diagnostics has proven difficult. This requires many topics surrounding quality assurance, assay validity, data security, legal and regulatory considerations and the interface with pre-existing hospital infrastructure all to be addressed. Many molecular diagnostics departments run in-house developed bioinformatic and variant interpretation pipelines, with wide variation in the extent to which comparisons against gold standards and proficiency testing are performed. An alliance of Nordic laboratories implementing genomics in clinical settings, the Nordic Alliance for Clinical Genomics, has applied the approach of peer-driven collaboration to a series of benchmarking exercises. Components of the NGS diagnostic pipeline including variant calling, variant interpretation and clinical reporting were all examined in turn. Benchmarking exercises were designed by members of the alliance to reflect their real-world needs, then executed, before the results were discussed in plenum at a series of bi-annual workshops. 6 workshops have been held to date, with the number of participants growing from 18 to 66 at each workshop, and the corresponding number of participating organizations

from 5 to 19. This approach gave the participating units the unique opportunity to compare their production performance against each other, and for differences and findings to be discussed on a practical level and summarized. For all three exercises, one or more participating units incorporated learnings and implemented changes in their current clinical pipelines, demonstrating the value of a peer-driven forum for such quality improvement activities.

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P14.017D

Next Generation Sequencing (NGS) as a key player in improving diagnostic yield for rare diseases (RDs): the collaborative experience of 2 centres in Greece

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Introduction: We report 12 months of clinical NGS by the Department of Medical Genetics, Athens University, and a collaborating service lab, for patients referred for a wide range of genetic conditions.

Material and Methods: During 12 months, 186 patients (85% pediatric) were referred, following clinical evaluation, pre-test counselling and signed informed-consent. Clinical geneticists and other medical specialists selected whole or clinical exome sequencing (~19,000 or ~4,500 genes) according to phenotype. Library preparation used Whole Exome Solution and Clinical Exome Solution kits (Sophia Genetics), run on a NextSeq-500 (Illumina). Bioinformatics analysis used SOPHiA DDM® and VarAFT 2.14. Variants were categorised according to ACMG guidelines; only "Pathogenic" or "Likely pathogenic" variants were reported. In most cases Sanger sequencing confirmed variants and family segregation.

Results: RDs included: Neurodevelopmental (28 patients), Neuromuscular (31), Skeletal/connective tissue (20), Metabolic (8), Dysmorphic syndromes (16), Intellectual disability/Autism (6), Kidney (10), Cardiopathies (10), Skin (6), Ears/Eyes (11), Other (30). Diagnostic yield was 42% overall (78/186 cases), reaching >60% for Neurodevelopmental, Skeletal/connective tissue, Skin, Ears/Eyes; 56 cases exhibited autosomal dominant inheritance (49 de-novo, 7 inherited), 12 recessive and 10 X-linked, including 35 known, 47 novel variants (submitted to ClinVar) and 8 variants of uncertain significance (VUS).

Discussion: NGS supports marked improvement in diagnostic-yield for many RDs, sometimes revealing atypical phenotypes and/or genotypes. Higher diagnostic yield is achieved for clinically well-defined cases, supported by family segregation studies. Close collaboration between the laboratory and clinics, supports high quality standards (clinical, laboratory, interpretation, counselling/reporting), fundamental to optimise definitive diagnosis, although VUS remain challenging.

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P14.018A

Utility of gene panel testing in children with seizure onset after 2 years of age: Results from a European and Middle Eastern epilepsy genetic testing program

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Background: Epilepsy is one of the most common childhood-onset neurological conditions with a genetic basis. Genetic diagnosis provides potential for etiologically-based management and treatment. Existing research has focused on early-onset (<2 years) epilepsies while data regarding later-onset epilepsies is limited. Program goals: Determine, in a selected pediatric epilepsy cohort, the overall and actionable molecular diagnostic (MDx) yield and the CLN2 disease MDx yield. CLN2 is a severe, rapidly progressive neurodegenerative disease with onset of seizures at/after 2 years and average age-of-diagnosis of 5 years.

Methods: Blueprint Genetics' next-generation sequencing (NGS)-based 283-gene epilepsy panel was used. Copy number variant (CNV) detection from NGS data was included. Variant interpretation was performed according to ACMG guidelines. Program results (Oct/2017-Nov/2018) are reported from 210 patients (Europe, Middle East) with inclusion criteria: Age 24-60 months, first seizure at/after 24 months, and at least one additional finding. The program was sponsored by BioMarin Pharmaceutical Inc.

Results: Median age-at-testing: 42 months; median age-of-first-seizure-onset: 30 months; average delay from first seizure to comprehensive genetic testing: 10.3 months. Genetic diagnosis was established in 42 patients; 20.0%

MDx yield. CNVs were reported in 26.2% of diagnosed patients; 27.3% of CNVs identified were intragenic. MDx included 5 CLN2 (*TPP1* gene) diagnoses, 4 MECP2, 3 SCN1A, 3 Angelman syndrome, 2 each of CHD2, KCNA2, MFSD8, SCN2A and STXBP1.

Conclusion: This program demonstrates the clinical utility of a comprehensive epilepsy gene panel for patients with first seizures at/after 2 years for MDx of pediatric epilepsy and CLN2 disease to guide management and treatment.

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P14.019B
Single nucleotide polymorphism (SNP) based chromosomal microarray analysis may detect early stages of malignancy

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Chromosomal microarray analysis (CMA) detecting constitutional copy number variations (CNVs), is the first-tier test for individuals with developmental delay or congenital anomalies. We present a patient with acute myeloid leukemia (AML) incidentally diagnosed by CMA done on peripheral blood. A nine years old boy of consanguineous parents was referred to CMA because of multiple CAL spots with axillary freckling, and severe failure to thrive (weight and height: -3.5SD, head circumference: -4SD). At age four years he had Wilms tumor, with a relapse at age six. Former genetic workup included normal karyotype, methylation pattern of H19 and NF1 sequencing. SNP-based CMA testing revealed multiple changes in mosaic state: mosaic duplications of 1q, 3q, 6p, 21q, 11q, 21q, mosaic loss of 6p, and mosaic copy neutral loss of heterozygosity (CN-LOH) of 10q, 19q, 21q, with multiple homozygous regions (22%). This CMA result indicated hematological malignancy, later diagnosed as therapy related AML. Peripheral blood karyotype detected multiple spontaneous chromosomal changes, including: single and double strand breaks, quadri-radial structures, marker chromosomes and translocations. Diepoxybutane-induced breakage test did not indicate increased breakage. Bone marrow karyotype showed 6p deletion, partial trisomy of 11q, mosaicism for del5q, consistent with t-AML. Whole exome sequencing revealed a homozygous missense mutation in BLM (c.3416G>C), indicating Bloom syndrome, explaining the clinical presentation. Here we demonstrate that in addition to diagnosis of deletion/duplication syndromes, mosaicism and CN-LOH, the use of SNP-based CMA platforms allows detection of previously undiagnosed systemic maladies. We urge CMA analysts to be alert to changes in SNP pattern.

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P14.020C
Identifying rare copy number variants using genome-wide array data

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Introduction: Genotyping experiments by the Breast Cancer Association Consortium (BCAC) have identified more than 100 loci associated with the risk of breast cancer. Rare deletions and duplications within susceptibility genes are known to increase breast cancer risk but the contribution of rare CNVs within other genes and non-coding regions has not been studied with large sample sizes.

Materials and Methods: We called CNVs from the intensity measurements from Illumina custom arrays (iCOGS and Oncoarray) for ~150,000 breast cancer cases and ~120,000 controls. To reduce noise we applied a principal component adjustment to the intensities and strict sample and locus quality control. We calculated z-scores from the distribution of intensities at each probe and used circular binary segmentation to identify runs of probes where the z-scores for a sample were shifted. We derived cut-offs for the mean z-scores of segments that indicated probable deletions or duplications.

Results: Comparison with known rare CNVs in 1000 Genomes samples indicate a sensitivity rate of approximately 86% for deletions and 71% for duplications. We estimated the rate of false positive CNV calls to be around 7% for deletions and 16% for duplications. The z-scores

provide a metric for the confidence of the CNV call. Tests for breast cancer risk showed the strongest associations for known CNVs in CHEK2 and BRCA1.

Conclusions: By accounting for the variance in probe intensity on genotyping arrays, our method improves the calling of rare CNVs and may provide more powerful tests of disease association.

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P14.021D

A universal simple and cost-efficient digital PCR method for the targeted analysis of copy number variations

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Introduction: Rare copy number variations (CNVs) are a major cause of genetic diseases. Simple targeted methods are required for their confirmation and segregation analysis. We developed a simple and universal CNV assay based on dPCR and universal Locked Nucleic Acid (LNA)-hydrolysis probes.

Methods: We analyzed the mapping of the 90 LNA hydrolysis probes from the Roche Universal Probe Library (UPL). For each CNV, selection of the optimal primers and LNA probe is almost automated, probes are reused across assays and each dPCR assay includes the CNV amplicon and a reference amplicon. We assessed the assay performances on 93 small and large CNVs and performed a comparative cost-efficiency analysis.

Results: UPL-LNA probes presented nearly 20,000,000 occurrences on the human genome and were homogeneously distributed with a mean interval of 156 bp. The assay accurately detected all the 93 CNVs, except one (<200 pb), with variation coefficients below 10%. The assay was shown to be more cost-efficient than all the other methods.

Conclusion: The universal dPCR CNV assay is simple, robust and cost-efficient as it combines a straightforward design allowed by universal probes and endpoint PCR, the advantages of a relative quantification of the target to the reference within the same reaction, and the high specificity of the LNA-hydrolysis probes. This method should be a useful tool for genomic medicine, which requires simple methods for the interpretation and segregation analysis of genomic variations.

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P14.022A

Prenatal CNV detection by means of exome sequencing instead of microarray diagnostics: one test fits all!

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Introduction: Nowadays, in fetuses with structural abnormalities, rapid whole exome sequencing (WES) is frequently performed in addition to routine diagnostics tests (QF-PCR and microarray analysis). Since our in-house diagnostic workflow now allows for prenatal WES results within two weeks, we evaluated whether genome-wide, exome-based CNV analysis on prenatal samples with a non-aberrant QF-PCR result is sufficiently robust and accurate to replace the current microarray workflow.

Methods: For all samples received between 01/2016 and 10/2018 for which both rapid WES and microarray analysis were performed, aberrant microarray profiles explaining the ultrasound abnormalities were compared to exome-based CNV profiling data. In addition, for all clinically relevant CNVs reported in the prenatal setting in 2018, the potential detection of these using exome sequencing data, based on CNV size and/or gene content, was investigated.

A total of 21 clinically relevant CNVs (with sizes ranging from 500 kb – 8.7 Mb) were examined.

Results: Exome-based CNV analysis detected 21/21 of the investigated chromosomal aberrations, including

recurrent pathogenic CNVs such as the 22q11 microdeletion. The advantage of simultaneously detecting both SNVs and CNVs by WES is underscored by a fetus in which a paternal 1q21.1 microdeletion and a maternal SNV in the *RBM8A* gene were identified, resulting in autosomal recessive TAR syndrome.

Conclusions: Our results show that exome-based CNV profiling can detect clinically relevant copy number variations in prenatal samples that are also identified by microarray analysis. Exome-based CNV analysis can, therefore, replace prenatal microarray analysis for samples with a non-aberrant QF-PCR result.

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P14.023B
Low-coverage WGS with CNV detection is a viable replacement for aCGH

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Introduction: Copy number variations (CNV) are repeated sequence segments of DNA in the human genome which contribute substantially to normal population variability. However abnormal CNVs are a cause of numerous genetic disorders. Several methods for CNV analysis are used, from the conventional cytogenetic analysis through microarray-based methods (aCGH) to next-generation sequencing. We present GenomeScreen - NGS based CNV detection method.

Materials and Methods: A previously described CNV detection algorithm used for NIPT was adjusted to allow replacement of aCGH for whole blood or buffy coat. We determined theoretical limits of its accuracy and confirmed it with extensive in-silico study as well as genotyped samples.

Results: When using WGS achievement of theoretical limit for Z-score of >7, at least 3M uniquely mapped reads are required to detect deviation with the length of 200k bases or more. This finding is supported both with the in-silico analysis and analysis on lab-prepared samples. In both analyses we were able to detect all of the simulated deviations with length of at least 200k and even some with smaller length (between 100k and 200k).

Conclusions: We compared clinical samples defined by aCGH method in Slovakia (Human Genome CGH Microarray Kit, 4x44K), which has roughly 200k bases resolution. This resolution is in line with GenomeScreen. The disadvantage of the aCGH method are huge gaps longer than 200k bases leaving 38% of genome uncovered, whereas only 13% is uncovered when GenomeScreen is used (mainly centromeres). Lastly, cost per sample is about 2.5 times lower for GenomeScreen.

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P14.024C
Reproducibility of genome-wide CNV analysis and new concept for distant relatedness determination

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Introduction: High resolution microarray technology is widely used to detect CNVs. Interpretation of results is based on correct evaluation of the extent and clinical significance of each detected variant. Due to low population frequency of many benign variants, we decided to explore a new concept - application of CNV detection for distant relatedness determination. We analysed a large four-generation pedigree without any genetic impairment, we followed the segregation of CNVs and reproducibility of their calls.

Materials and Methods: We tested the performance of Agilent Sure Print G3 Human Microarray 2x 400K. We isolated DNA from saliva using Oragene technology. Agilent Male and Female Control DNAs were used.

Results: We compared 27 pairs of first degree relatives, 12 pairs of first cousins, 12 pairs of "uncle -nephew type"(r=1/4), 4 pairs of double first cousins and 15 pairs of second cousins. We followed three times the segregation from a common ancestor to the fourth generation and four times to the third generation. Typically we detected around 30 CNVs per individual and we followed the segregation with high precision (identical genomic coordinates for the analysed CNV in different individuals).

Conclusions: In our pilot study, we provided the evidence that a microarray with evenly distributed probes is able to detect segregation of CNVs with high reproducibility across four generations and that more efficient CNV detection algorithm based on WGS and appropriate population data may represent a handy tool for distant relatedness determination. Supported by the Ministry of Interior of the Czech Republic grant no.VI20172020102.

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P14.025D

Detection of copy number variations from NGS data using read depth information: a diagnostic performance evaluation

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Introduction: The detection of Copy Number Variations (CNVs) from NGS data is under-exploited and chip-based technologies or targeted techniques are still commonly used for their detection in a diagnostic setting. We assessed the performances of CNV calling using CANOES, a read-depth comparison method applied to gene panels and whole exome sequencing (WES).

Methods: We applied CANOES to NGS data obtained from (i) 465 samples with both gene panel and comprehensive Quantitative Multiplex PCR of Short Fluoresent (QMSPF) data available (total of 60 exons assessed), (ii) 95 additional samples with NGS data from 2 different gene panels, (iii) 135 samples with both WES and array CGH (aCGH) data available and (iv) 1,056 additional WES.

Results: From the gene panel data, CANOES detected all 14 events that were previously identified by QMPSF, with neither any false positive nor any false negative among 465 samples (Sensitivity (Se) = specificity = 100%). In addition, CANOES detected 97 candidate CNVs in 95 additional samples, 86 of which were confirmed by a targeted technique (PPV= 90.1% overall). From the WES data, CANOES detected 159 of the 195 exonic events previously detected by aCGH among the 135 samples with WES+aCGH data available (Se=81.5%). Overall, the PPV of CANOES from WES data was 94.8% after the confirmation of 108 of the 123 calls targeting a list of 355 genes among 1,056 additional patients.

Conclusions: CANOES showed very high diagnostic performances in the context of NGS gene panels. Combination with other detection tools may increase the diagnostic performances.

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P14.027B

Development of a genetic risk score for coeliac disease and validation in a clinical diagnostic setting

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Introduction: There has been increasing interest in genetic risk scores (GRS) in disease diagnosis. Specific HLA-DQ alleles predispose to coeliac disease (CD) and HLA typing is occasionally used as a rule-out test. However, CD is polygenic and genome wide association studies (GWAS) have implicated ~40 additional loci. Using single nucleotide polymorphisms (SNPs) we aimed to combine all associated loci into a GRS and assess its utility as a clinical tool.

Methods: We used imputation to identify SNPs strongly correlated(r^2>0.95) with 4 key HLA-DQ haplotypes (DQ2.5/DQ2.2/DQ7.5/DQ8) in UKBiobank. We derived HLA-DQ odds ratios from 12,000 cases and 12,000 controls(Wellcome Trust). We combined this with additional SNPs from recent GWAS to generate a coeliac genetic risk score(C-GRS).We validated the C-GRS in a population based cohort(UKBiobank) with 1237 cases identified by hospital admission codes. We genotyped the C-GRS in 161 samples from a paediatric clinic where patients had been assessed using anti-tissue transglutaminase antibodies, biopsy and HLA typing.

Results: The C-GRS consisted of 42 SNPs and was highly discriminative of CD in UKBiobank. The C-GRS was more discriminative than HLA stratification alone (ROC-AUC=0.88 [95%CIs:0.87-0.89] v 0.81, p<0.0001) and highly discriminative in the paediatric clinic (ROC-AUC=0.82 [95%CIs:0.75-0.90], p<0.0001).

Conclusions: A C-GRS can aid in identifying incident cases of CD and is more effective than HLA typing alone. Given the low costs of SNP genotyping relative to HLA typing a C-GRS could improve the availability and utility of coeliac genetic testing in CD diagnosis and in recruitment to research studies.

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P14.028C
Clinical validation of Copy Number Variant (CNVs) detection by Next-Generation Sequencing (NGS)

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Objectives: Despite the great advances achieved in clinical genetics enabled by the incorporation of Next-Generation Sequencing (NGS), a significant percentage of patients with genetic diseases still do not have a conclusive molecular diagnosis. The incorporation of specific pipeline bioinformatic methods has allowed the implementation of Copy Number Variants (CNVs) detection in NGS analysis, improving its diagnostic efficiency. In this study, the clinical utility of the detection of CNVs by NGS has been proven.

Materials and Methods: During 2018, 275 patients were studied using the NGS technique without obtaining an accurate genetic diagnosis.

Bioinformatic tools that compare the normalized sequencing depth between patients and controls were used to determine CNVs. The results obtained were compared with patients owns laboratory database. CNVs were confirmed by Multiplex Ligation-dependent Probe Amplification (MLPA).

Results: Pathogenic CNVs causing the disease were detected in 11 out of the 275 patients (4%). Specifically, CNVs were detected for pathologies with autosomal dominant inheritance patterns (*TSC2*, *MSH2* and *FBN1*), as well as for genes with autosomal recessive inheritance patterns, including two homozygous deletions (*KCNV2* and *RDX*) and one heterozygous deletion with a *Single Nucleotide Variant* (SNV) in the *PKHD1* gene. One of the most notable cases corresponds to a patient suspected of hypomagnesemia who showed compound heterozygous deletions in the *TRPM6* gene.

Conclusion: These results confirm that the inclusion of the detection of CNVs by NGS in the genetic diagnostic routine allows to increase the diagnostic efficiency offered, obtaining increasingly significant results and with a better cost-effectiveness than conventional techniques.

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P14.029D
Evaluation of preliminary benchmark deletions for the reference sample NA12878

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Introduction: Reference materials and standards play a pivotal role in research and clinical settings alike for benchmarking the quality of genetic material used, data produced and performance of software tools used for bioinformatic analyses. Deficiencies in the baseline truth set will result in misleading performance metrics. In this work,

we evaluated the preliminary benchmark deletions published by the genome in a bottle consortium (GIAB) for the reference sample NA12878.

Methods: We used orthogonal data from Illumina SNP array, Illumina short-read sequencing and long-read sequencing data from PacBio and Nanopore to manually review every deletion greater than 1kb in size. To assess the presence of each deletion call, we computed the relative read depth across the deletions compared to the rest of the genome, evaluated paired-end, and split-reads supporting the deletion call.

Results: There were 610 deletions (>1kb) in the preliminary benchmark dataset published by the GIAB. Fifteen percent of the deletions (94/610) were found to be LINE insertions in the reference genome falsely identified as deletions in the NA12878. Sixteen percent of the deletions (98/610) had conflicting evidence across datasets. We saw evidence for 69% of the deletions (423/610) across datasets.

Conclusion: We performed a manual review of 610 preliminary benchmark deletions dataset for the sample NNA12878. We found 31% of the variants were either to be likely false-positive or ambiguous across orthogonal platforms. This is the first important step in understanding the false positive deletions from genome sequencing data and refine the existing benchmark dataset.

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P14.030A

Detection of genetic imbalances by array-based comparative genomic hybridization in patients with syndromic craniostenosis

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Introduction: Craniosynostosis is characterized by the premature fusion of one or several of the calvarial sutures. This fusion restricts normal growth of the skull, face and brain causing a number of neurological and neurosurgical complications. Craniosynostosis can be isolated or syndromic. Syndromic craniostenosis is usually caused by a genomic imbalance or deficit although the exact mechanism oftenly remains unknown. Array-based comparative genomic hybridization (array CGH) is a powerful and high-resolution approach for detection of DNA copy number variants (CNVs).

Materials and Methods: The genome profiling of forty-two patients with syndromic craniosynostosis was carried out by oligo array CGH. Agilent ISCA, 4x44, v2.0, with 35 kbp backbone resolution were used. The slides were scanned on Agilent fluorescent scanner G2505C and analyzed by BlueFuse Multi, v 4.2 (20289) (BlueGnome, Cambridge, UK).

Results: We found five pathogenic rearrangements consisting of: three deletions (ranging from 1.1 to 11.24 Mb) and two duplications (ranging from 14.92 to 25.19 Mb). We also found two likely pathogenic mutations: one duplication (4.62 Mb) and one deletion (177.93 Kb).

Conclusion: Finally we concluded that 16.6 % of our patients carry clinically significant, pathogenic and/or likely pathogenic mutations. These data strongly support the idea that only a whole-genome high-resolution analysis such as array CGH is able to provide an accurate diagnosis for genetic imbalances in patients with syndromic craniostenosis.

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P14.031B

CRISPR/Cas9 mediated enrichment of targeted genomic regions in human

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Introduction: CRISPR/Cas9 technology has been widely used for different modification of eukaryotic and bacterial genomes. Here we applied (inactive) Cas9 for guided enrichment and hence targeted sequencing of human genomic regions in proximity of *LDLR* (chr19), *PCSK9* (chr1) and *APOB* (chr2), genes associated with the familial hypercholesterolemia.

Materials and Methods: Specific single guide RNA (sgRNA) probes were constructed and used for targeting *LDLR*, *PCSK9* and *APOB* genes in human DNA, extracted from whole blood samples. Different approaches encompassing SNAP-tag, biotin-tag, and immunoprecipitation of (inactive) Cas9 enzyme, were used for capturing of double-stranded DNA target regions in fragments of app. 30k bp in size. Captured DNA was further used for Illumina library preparation, sequencing and downstream bioinformatics analysis.

Results: Applying different ratios between sgRNA, human DNA, and dCas9 enzyme, together with multiple capture techniques, resulted in successful enrichment,

especially when using biotin based capture and molar excess of sgRNA/Cas9. Control and reduction of off-target DNA/Cas9 associations and unspecific binding of DNA to paramagnetic particles used for immunoprecipitation emerged as the crucial parameters affecting the enrichment efficiency.

Conclusion: Altogether, our approach offers additional insight into specific enrichment of targeted genes and presents cost-effective and rapid tool in the investigation of the specific genomic regions associated with familial hypercholesterolemia. Grant information: Tertiary Projects, Medical University Centre of Ljubljana (Nr. 20170085).

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P14.032C
Breast cancer circulating tumour cell (CTC) detection and characterization using a bead-based RNA multiplex panel

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Introduction: The presence of ≥5 circulating tumour cells (CTCs) per 7.5 ml of blood correlates with poor prognosis in metastatic breast cancer patients. Molecular characterisation of CTCs requires highly sensitive assays capable of detecting low-frequency RNA transcripts.

Method: A QuantiGene™ 4-plex assay was optimized for direct use on lysed CTCs, to quantitatively assess molecular biomarkers associated with breast cancer classification (*ERBB2*), epithelial CTC detection (*EPCAM*, *KRT19*) and epithelial-mesenchymal transition (*FN1*). Serial dilutions (5-1000 cells) of five breast cancer cell lines known to express at least one of the target genes (SKBR3, BT474, JIMT-1, MCF7 and Hs578T) were prepared and microscopically counted. The cells were lysed and used directly in the QuantiGene™ 4-plex assay. Readings were taken on the Luminex® 200™ and MAGPIX® instruments.

Results: The assay gave linear readings ($R^2 \geq 0.99$) for the genes expressed by each cell line down to a mean limit of 26 cells (range: 6-58). *KRT19* outperformed *EPCAM* as epithelial biomarker. The mesenchymal, triple-negative cell line Hs578T expressed only *FN1*, which maintained a linear signal down to only 6 cells. In the HER2-positive cell lines, *ERBB2* was an excellent biomarker, even in JIMT-1, which is known to be a low expresser. High concordance ($R^2 \geq 0.87$) was recorded between the two instruments.

Conclusion: Direct cell lysis and signal amplification technology make bead-based multiplex RNA panels applicable on liquid biopsies in the clinical laboratory. The technique is being currently optimized with the use of upstream, antibody-independent CTC enrichment methods for its application in patient liquid biopsy specimens.

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P14.033D
Comparative validation of two molecular tag-based library systems for sequencing of circulating tumor DNA

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Liquid biopsy, the analysis of cell-free DNA released by tumor cells into plasma, is becoming a promising biomarker in clinical oncology. Potential applications include: optimizing therapy (e.g. detecting resistance), monitoring residual disease, appraising tumor mutation burden, and early detection of relapses. Yet, this analysis is technically challenging, largely because the minute amounts of circulating tumor DNA (ctDNA) often require detecting mutations at frequencies lower than 1%. Molecular tags are a recent technical improvement allowing to better distinguish low frequency mutations from sequencing noise.

With the aim of implementing novel diagnostic tests in our hospital, we systematically compared two leading commercial systems for molecular tags libraries: Avenio (Roche) and QiaSeq (Qiagen), both with synthetic control DNA (Horizon) and with DNA from patients with lung or colon carcinoma. Our comparison included a set of 12 cases of colorectal cancer for which we compared sequencing of liquid biopsies with that of the initial tumors. Overall, there was excellent agreement between the two ctDNA sequencing techniques, but poor correlation with the initial tumor, likely due to tumor evolution during the delay between surgery and liquid biopsy. Dilution experiments revealed excellent linearity of detection, with a lower limit of quantification around 0.2%. Repeatability and reproducibility were good. A key factor was bioinformatic analysis, and dedicated software proved superior to generic open-source programs.

In conclusion, both ctDNA sequencing systems proved equally good and provided sensitivities in the 10^{-3} range, thus approaching the theoretical limit that can be expected given the low amount of ctDNA in plasma.

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P14.034A
Effective neonatal genetic diagnostics of cystic fibrosis in Khanty-Mansi region (Russia)

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Introduction: Newborn screening (NBS) dramatically improved cystic fibrosis (CF) preclinical diagnostics, although the need of early detection of *CFTR* mutations still challenges regional genetics laboratories in Russia. We applied 3-step approach using known molecular methods for fast mutation search, which helped physicians quickly start appropriate neonatal treatment.

Materials and Methods: gDNA from 501 dried blood spots (DBS) obtained using column extraction; high resolution melting (HRM) analysis performed using "Precision Melt Software" (Bio-Rad); Sanger sequencing, MLPA performed on "GenomeLab GeXP" (Beckman Coulter) according to manufacturers' protocols.

Results: We applied 3-step approach (HRMA-Sanger-MLPA) for all suspicious CF cases after NBS. In 2016 - 2018 we studied 401 samples and revealed 34 carriers with one *CFTR* mutation and 17 children (later included in regional CF registry) with two mutations. First we looked for seven major regional mutations using HRM-based genotyping, then HRM-based gene scanning revealed exons with deviating melting patterns, which in turn were subjected to Sanger sequencing. Finally, MLPA was used for all samples. All findings (see Table) were validated later with 100% concordance by NGS performed elsewhere. This study also updated the list of major regional *CFTR* mutations.

Conclusions: the proposed approach allowed to perform *CFTR* mutation testing rapidly (TAT 3 - 4 days) for neonatal DBS samples at level of regional genetics laboratory, which is particularly important for new CF cases among scattered population in Siberia.

List of mutant alleles revealed in the study

№	HGVS	Legacy	mutant alleles number	% from total
1	c.1521_1523delCTT (p.Phe508del)	[delta]F508	44	64.6
2	c.274G>A (p.Glu92Lys)	E92K	4	5.9
3	c.54-5940_273+10250del21kb (p.Ser18ArgfsX16)	CFTRdele2,3	3	4.4
4	c.412_413insACT (p.Leu137_Leu138insThr)	L138ins	2	2.9
5	c.1399C>T (p.Leu467Phe)	L467F	2	2.9
6	c.1545_1546delTA (p.Tyr515X)	1677delTA	2	1.5
7	c.3196C>T (p.Arg1066Cys)	R1066C	2	1.5
8	c.43delC (p.Leu15PhefsX10)	175delC	1	1.5
9	c.489+1G>T	621+1G->T	1	1.5
10	c.653T>A (p.Leu218X)	L218X	1	1.5
11	c.1040G>A (p.Arg347His)	R347H	1	1.5
12	c.1624G>T (p.Gly542X)	G542X	1	1.5
13	c.2012delT (p.Leu671X)	2143delT	1	1.5
14	c.3208C>T (p.Arg1070Trp)	R1070W	1	1.5
15	c.3846G>A (p.Trp1282X)	W1282X	1	1.5
16	c.3983 T>A (p.Ile1328Lys)	-	1	1.5
			68	100

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P14.035B
Newborn screening EQA for Cystic fibrosis (CF) and Medium-chain acyl-CoA dehydrogenase deficiency (MCADD): Ten year review (2008-2018)

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Newborn screening is performed within the first 8 weeks of life to screen for diseases which are treatable if detected early. Most of these tests are biochemical based but two disorders include molecular testing for confirmation of diagnosis; Medium chain acyl-CoA dehydrogenase deficiency (MCADD) and cystic fibrosis (CF). To monitor the ability of molecular laboratories to detect MCADD and CF mutations from neonatal blood spot cards, an external quality assessment (EQA) was set up in 2008. For these EQAs, samples are distributed four times a year to provide continual monitoring of the quality of testing. To mimic the newborn screening carried out in laboratories as closely as possible, routine neonatal bloodspot cards are used with real patient blood samples. For the CF EQA a variety of different CF variants are tested including the four most common European *CFTR* variants (p.Phe508del, p. Gly542*, p.Glu551Asp and c.621+1G>T). Compound heterozygotes and samples in which no variants are detected are also included. For the MCADD EQA, participants are requested to test the samples for the most common *ACADM* variant c.985A>G p.(Lys329Glu) Approximately 30 laboratories currently participate in one or both bloodspot EQAs. These include participants from nine countries. A review of these EQAs over the last ten years will be presented.

Including participating countries, the samples used in the EQA runs, common errors and changes in mutation nomenclature.

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P14.036C
Next-generation cytogenetics in medical genetics with high-resolution optical mapping

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Structural variants (SVs) are an important source of genetic variation in the human genome and they are involved in a multitude of human diseases, including cancer and developmental disorders. In a diagnostic set-up, comprehensive analysis of all molecular cytogenetic aberrations in a given sample still requires a combination of techniques, such as CNV-microarrays, karyotyping and FISH. We hypothesize that the combination of classical approaches could be largely replaced by novel optical mapping technology.

We had early access to the new SaphyrDC system (BioNano Genomics), which enables SV detection based on optical mapping of labeled high-molecular weight DNA. We performed optical mapping with >100X genome coverage to detect cytogenetic aberrations in 4 leukemia samples and compared the results with those obtained using classical methods. In all samples, a very good concordance with the results was achieved. This held true for deletions, insertions, inversions and translocations, including 3-way Philadelphia chromosome (46,XX, t(9;22;14)(q34;q11;q11.2)) and even chromothripsis. Importantly, we were able to identify aberrations in samples with a cancer cell content of just ~40%, using the latest single-molecule SV detection tool from BioNano. Optical mapping also identified novel events e.g. an inversion of chromosome 11 (chr11:24,875,044-26,299,641) and a translocation (t(5;14)(q35.2;q 32.2)), both validated afterwards.

Optical mapping may have the potential to replace most classical cytogenetic tests. Therefore, we are now launching a study to systematically compare the sensitivity and specificity of optical mapping in 100 leukemia samples and 50 samples with known germline cytogenetic aberrations against the standard of care workflow.

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P14.037D
Identification of genomic aberrations in children with global developmental delay or intellectual disability through detecting copy number changes and whole exome sequencing

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Developmental delay (DD) is the late emergence of developmental milestones during infancy and early childhood. Copy number variations (CNVs) have been regarded as one of the major causes of DD. The aim of this study is to determine the efficiency of whole exome sequencing (WES) as a first-tier diagnostic test in comparison with array comparative genomic hybridization (aCGH) for DD.

We have enrolled 322 subjects diagnosed with global developmental delay or intellectual disability of unknown cause. All individuals have completed aCGH tests (Positive rate: 17.1%). WES was performed on 24 trios and 10 probands with Agilent SureSelect target enrichment system using Illumina HiSeq2000. Twelve trios have been analyzed by Golden Helix Varseq. On average, 339 CNVs with confirmed states, absent quality control flags, and p-values<0.01 were called. Spans of these CNVs ranged from 118bp to 2Mbp, with approximately 329 CNVs <100kb, 9 CNVs 100kb-1Mb, and 1 CNV >1Mb in each case. Duplications accounted for 57.6% confident CNV calls. Moreover, WES allows us to investigate single nucleotide variations (SNVs). Among the 12 trios, we have discovered causal SNVs for two cases corresponding with their clinical phenotypes. Further validation by qPCR is required to confirm the precision of CNV detection results by WES. We will also evaluate how consistent WES detection results are with aCGH.

Here we demonstrate the capacity of WES for detecting CNVs and SNVs. WES may be considered a promising first-tier diagnostic tool for its versatility, moderate cost, and the potential to reduce diagnostic odyssey.

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P14.038A
A head-to-head evaluation of the diagnostic efficacy and costs of trio versus singleton exome sequencing analysis

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Diagnostic exome sequencing (ES) can be performed on the proband only (singleton; sES) or proband and both biological parents (trio; tES). In this study we sought to compare the efficiencies of exome sequencing (ES) by trio (tES) versus singleton (sES) approach, determine costs, and identify factors to consider when deciding on optimal implementation strategies for the diagnosis of monogenic disorders. We undertook ES in 30 trios and analysed each proband's sES and tES data in parallel. Two teams were randomly allocated to either sES or tES analysis for each case and blinded to each other's work. Each task was timed and cost analyses were based on time taken and diagnostic yield. We modelled three scenarios to determine the factors to consider in the implementation of tES. sES diagnosed 11/30 (36.7%) cases and tES identified one additional diagnosis (12/30 (40.0%)). tES obviated the need for Sanger segregation, reduced the number of variants for curation, and had lower cost-per-diagnosis when considering analysis alone. When sequencing costs were included, tES nearly doubled the cost of sES. Reflexing to tES in those who remain undiagnosed after sES was cost-saving over tES in all as first-line. This approach requires a large differential in diagnostic yield between sES and tES for maximal benefit given current sequencing costs. tES may be preferable when scaling up laboratory throughput due to efficiency gains and opportunity cost considerations. Our findings are relevant to clinicians, laboratories and health services considering tES over sES.

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P14.039B

Analytical validation of a sensitive myotonic dystrophy type 1 (DM1) diagnostic test that provides precise repeat sizing and resolves zygosity in a single PCR

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Introduction: Myotonic dystrophies are autosomal dominant, multisystemic disorders with variable expressivity characterized by progressive myopathy. Myotonic dystrophy type 1 (DM1) is caused by CTG expansions in the 3' UTR of the *DMPK* gene on chromosome 19q13.3. The severity of disease and age at onset is roughly correlated with the number of the trinucleotide repeats. Reliable assessment of CTG expansions is critical for diagnosis of DM1. We developed and validated the AmplideX® DM1 Dx Kit, which generates numerical values for alleles ≤200 repeats and a categorical value for alleles >200 repeats.

Materials and Methods: The kit enables repeat-primed PCR of genomic DNA isolated from whole blood, followed by capillary electrophoresis (CE), manual peak annotation, and automated repeat length calculations. For exploratory size estimation beyond 200 repeats, the kit facilitates an optional agarose analysis.

Results: Analytical validation spanned 2192 valid measurements, 1588 genotypes, and 730 unique CE files. Single-site precision demonstrated that alleles with 5 to 145 repeats were sized within 1 repeat of the mode (n>200). The limit of detection was <4% fractional mosaic by probit analysis. All homozygous and heterozygous samples were correctly identified. Expanded alleles were accurately sized when ≤200 repeats and larger expansions up to at least 1900 repeats were amplified and categorically detected. A method comparison yielded highly correlated results (100% concordance and y=0.91+1.01x, R2=1.00).

Conclusions: This single-tube assay combines gene-specific and repeat-primed designs to assess DM1 genotype and resolve zygosity. It is a streamlined procedure that reduces the labor and turnaround time required for southern blot.

G. Latham: A. Employment (full or part-time); Significant; Asuragen. **K. Jefferson:** A. Employment (full or part-time); Significant; Asuragen. **J. Kemppainen:** A. Employment (full or part-time); Significant; Asuragen. **V. Le:** A. Employment (full or part-time); Significant; Asuragen. **J. Wisotsky:** A. Employment (full or part-time); Significant; Asuragen. **M. Fahey:** A. Employment (full or part-time); Significant; Asuragen. **B. Hall:** A. Employment (full or part-time); Significant; Asuragen. **J.T. Brown:** A. Employment (full or part-time); Significant; Asuragen.

P14.040C

Solution-based isolation of ultra-high-molecular weight (UHMW) DNA from fresh/frozen human blood and cultured cells in less than 3 hours

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Optical mapping of genomic DNA on the Bionano Genomics Saphyr® system for genome assembly or structural variation detection relies on starting with UHMW DNA. To achieve this, we developed methods for the isolation of genomic DNA that involves the embedding of material in agarose plugs, and overnight lysis *in situ* with detergent and proteinase K. After extensive washing on day 2, the agarose plug is melted, treated with beta agarase, and the liberated DNA drop dialyzed. The UHMW DNA is ready for quantification and labeling on day 3. This "plug lysis" method is extremely robust, but it is also labor intensive, difficult to automate, lengthy and expensive. To address these shortcomings, we coupled solution-based lysis with a purification step that leverages a novel process to bind, wash and elute UHMW genomic DNA. This entire protocol can be conducted in less than 3 hours on a batch of 6 samples, allowing 12 samples to be processed in one day. The eluted material is ready to use by day 2 and contains high quality DNA that is clean enough for the direct label and stain (DLS) protocol. The resulting labeling metrics of this labeled DNA on a Saphyr Chip® are comparable to labeled DNA isolated by the traditional "plug lysis" protocol. We have validated protocols for fresh/frozen human blood and cells, and are developing protocols for plant and animal tissue. These protocols are automatable, providing the needed solution for researchers needing to purify DNA from hundreds to thousands of individuals per year.

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P14.041D
Turbolase: a radically streamlined high throughput sample prep to sequencing workflow

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Normalase is a novel enzymatic library normalization method that eliminates library quantification and manual concentration adjustment of each sample prior to library pooling. When combined with Swift 2S Turbo rapid library kits, a highly streamlined 'Turbolase' workflow is created that is readily automated on the Hamilton Star and other platforms, where simple bulk processing improves throughput and reduces cost for NGS laboratories. Swift 2S Turbo kits comprise two enzymatic steps and a single purification that completes DNA fragmentation, end repair and adapter ligation. This is followed by standard library amplification using Normalase PCR primers to condition the libraries and produce a required minimum yield in excess of the 4 nM final concentration. This is followed by a single purification and two 15-minute Normalase incubation steps that; 1) enzymatically select 4 nM of each library, and 2) enzymatically normalize each library to 4 nM within a single pool. The pools can then be directly sequenced without further purification. Normalase allows for > 10-fold variation in input quantity, while generating ≤ 10% variation in sample representation within a pool resulting in optimal cluster density and sample balance for Illumina sequencing. The 'Turbolase' workflow is compatible with full-length indexed adapters that have been added by ligation as well as workflows that require indexing PCR primers. Normalase is also compatible with library preparation kits available from other vendors. Normalase for pooling of libraries for pre-hybridization capture is currently under development, which make multiplexed hybridization capture protocols streamlined and robust from library generation to sequencing.

H. Fiske: A. Employment (full or part-time); Modest; Swift Biosciences. **D. Masser:** A. Employment (full or part-time); Significant; Swift Biosciences. **R. Stedtfeld:** A. Employment (full or part-time); Significant; Swift Biosciences. **J. Lenhart:** A. Employment (full or part-time); Significant; Swift Biosciences. **V. Makarov:** A. Employment (full or part-time); Significant; Swift Biosciences. **L. Kurihara:** A. Employment (full or part-time); Significant; Swift Biosciences.

P14.042A
New approaches for adapter synthesis in Duplex Sequencing

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Introduction: Duplex Sequencing (DS) is a next generation sequencing methodology capable of detecting ultra-low

mutation frequencies by exploiting the fact that genetic information is stored on complementary DNA strands. Therefore, DNA fragments are ligated to adapters containing a double stranded degenerate sequence. Currently, fork adapters consisting of two partially complementary oligonucleotides are being used in DS. However, it has been suggested that adapters comprising a hairpin loop structure, such as the NEBNext adapters, show improved performance in library preparation and sequencing. Here, we propose different synthesis methods for creating hairpin loop adapters for DS in house.

Material and Methods: We have developed a synthesis strategy for a hairpin adapter and for adapters containing a phosphorothioate bond at the ligation site. Currently we use an adapted protocol for DS library preparation with those loop adapters. Adapter synthesis and proper function has been verified by gel electrophoresis and sequencing.

Results: We have successfully synthesized the proposed hairpin adapters and replaced the fork adapter used in the original protocol by Schmitt et al. (2012) with our loop adapters. Sequencing data confirm their experimental feasibility.

Discussion: Our hairpin adapters might offer advantages over previously used fork adapters due to structural differences. Additionally, we integrated a phosphorothioate bond into DS hairpin adapters which might increase the stability of the ligation site, reduce removal of the 3' T-overhang and thus, decrease potential adapter dimer formation between blunt-ended sites.

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P14.043B

A gene-agnostic trio exome strategy maximises diagnostic yield by uncovering disease-causing variants in newly discovered disease genes

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Rare genetic diseases in patients with non-specific/heterogeneous disorders are difficult to diagnose and many patients go through a "diagnostic odyssey" of several years and multiple testing before a genetic diagnosis is confirmed.

Genomic testing holds the promise of timely diagnoses but the choice between numerous gene panels *versus* "whole" exome sequencing (WES) as a first line diagnostic test is not straightforward; cost, turnaround time, likelihood of success and the possibility of uncovering unsolicited findings are important considerations that leave many clinicians favouring gene panels over WES.

We analysed 601 consecutive trios referred for diagnostic testing using an in-house pipeline to filter rare potentially deleterious variants by mode of inheritance. Variant classification was as according to the ACMG-AMP guidelines and variants of interest were discussed in an MDT meeting.

A diagnosis was identified in 38.4% of cases (231/601) and in a further 4% (26/601) a candidate variant is under investigation. De novo variants explained 55% of the diagnoses with the remaining cases having a recessive (38%), X-linked (6%) or mitochondrial aetiology (1%). Parental mosaicism was rare (<1% of cases). A number of the diagnoses were in newly discovered genes where association with the disorder was published within <3 years of the referral.

We will discuss the limitations of a gene panel approach which in our series would leave 5-10% of the diagnoses unidentified. WES offers a higher diagnostic yield and allows for future data re-analysis to include newly discovered genes.

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P14.044C

Exome sequencing of pooled parental DNA: a cost-efficient "trio-like" strategy to increase diagnostic yield in developmental disorders

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Introduction: In intellectual disability and developmental abnormalities (ID/DA), clinical exome sequencing (cES) is efficient for identifying variants in known disease-causing genes with a diagnostic yield around 30% in singleton strategies that can increase to 40-50% with a trio-based strategy. Indeed, trio-based strategy facilitates variants interpretation thanks to parental segregation but increases sequencing cost. We so explored an alternative cost-efficient trio-like approach, based on parental DNA pooling.

Materials and Methods: We pooled 6 paternal and 6 maternal DNA into two separate mixes. ES was performed with an average depth of 70X (TWIG) or 100X (Agilent CREV2) and an expected allelic balance in the pool of 5.8% and 8.3%, respectively.

Results: After a conclusive proof-of-concept in 6 positive individuals, this pool strategy was initially applied in 29 individuals with DI and/or AD, as second-tier after normal singleton cES. We so identified candidate variants in 19 different genes. After datasharing, we confirmed causal implication of 7 genes in 8/29 individuals (27%) including 3 genes newly involved in human disorders. This high rate led us to deploy this strategy as a first-tier exam in 82 individuals without previous cES.

Conclusion: In ID/DA, parental pool strategy appears very cost-efficient by combining increased diagnostic yields compared to singleton ES and decreased sequencing costs, compared to classical trio approach. This could represent an interesting alternative to trio-based ES in molecular laboratories performing exome routinely.

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P14.045D
Establishing high sensitivity and specificity for exome sequencing on Ion Proton

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Library preparation for whole exome sequencing is a critical step serving the enrichment of the regions of interest. For Ion Proton, there are only two exome library preparation methods available, AmpliSeq and SureSelect. Although of major interest, a comparison of the two methods is *hitherto* missing in the literature. Here we systematically evaluate the performance of AmpliSeq and SureSelect and present an improved variant calling pipeline. We used 12 in-house DNA samples with genome-wide and exome microarray data and a commercially available reference DNA (NA12878) for evaluation. Both methods had a high concordance (>97%) with microarray genotypes and when validating against NA12878 a sensitivity and positive predictive value (PPV) of >93% and >80%, respectively. Application of our variant calling pipeline decreased the number of false positive variants dramatically by 90% and resulted in PPV of 97%. This improvement is highly relevant in research as well as clinical setting.

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P14.046A
Reanalysis of exome sequencing data from an Estonian cohort of 85 families with suspected Mendelian disorders

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Exome sequencing (ES) is a proven powerful tool to identify the molecular etiology of Mendelian disorders, with a diagnostic yield of 25-40%, 29.3% in our lab after initial diagnostic analysis. We performed reanalysis of ES data and trio genome sequencing (GS) data in unsolved Estonian families with suspected Mendelian disorders to increase the diagnostic yield. ES reanalysis was performed in 85 families (66 trios, 19 singletons) at Broad Institute of MIT and Harvard. Additionally, ten families with proband-only

ES were expanded to trio ES, and 39 ES-negative families were re-sequenced with trio GS at Broad Institute for improved variant detection. We identified a pathogenic variant in a known disease gene in 14 (16%) cases; these were mainly missed due to: limited ability to detect *de novo* variants in proband-only ES; bioinformatics issues in early ES studies; or analysis being executed before the relevant disease gene discovery. In 15 (18%) families, we identified a pathogenic variant in a novel disease gene, of which some are already published via collaborations initiated through the Matchmaker exchange (*RORA*, *RAB11A*, and *CYFIP2*). In 27 (32%) families a candidate gene was found and functional studies are in progress for eight of these. The remaining 29 (34%) patients are still unsolved. Using this approach, we improved our diagnostic yield for previously undiagnosed cases by solving an additional 1/3 of the cases (total 53%) and by having a promising gene candidate for another 1/3 of the remaining families. Funding: Estonian Research Council grants PUT355, PRG471, and PUTJD827.

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P14.047B

Initiative for the harmonization of the quality assessment for analyses performed by the Belgian Centers for Human Genetics in the context of Rare Diseases

J. Lantoine[1], V. Benoit[2], A. Brysse[3], K. Claes[4], A. Corveleyn[5], M. De Rycke[6], V. Dideberg[3], E. Fastré[7], L. Van Laer[8], M. Ravoet[7], S. Rombout[2], C. Rydlewski[9], S. Seneca[6], S. Symoens[4], K. Van Den Bogaert[5], F. Wilkin[9], W. Wuyts[8], P. Van De Walle[1], N. M. Vandevelde[1]

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The Belgian healthcare authorities want to support the Belgian Centers for Human Genetics (BCHGs) in the development of a Quality System and participation to External Quality Controls (EQAs). One of the objectives of the Belgian Plan for Rare Diseases is to develop funding for their participation to EQAs for genetic tests focused on hereditary rare diseases.

A screening of quality controls focused on rare diseases available in Belgium and abroad was firstly performed. Based on this preliminary step, a survey was sent to the BCHGs to collect information about their participation to these inventoried quality controls, their provider and their evaluation of the schemes. Then, a working group (WG) composed of members from the BCHGs and the Belgian institute for health has been set up.

This working group is selecting priority EQAs for which participation fees should be covered by the Belgian healthcare authorities according to clinical relevance and costs.

Since there is no rules, the WG is also focusing on the harmonization of the participation frequencies to EQAs by writing guidelines on the minimal frequency of participation to EQAs focused on hereditary rare diseases' diagnosis.

These guidelines will provide recommendations on how often it is necessary for the BCHGs to perform EQAs and they will serve as basis for the reimbursement of the selected EQAs. The proposed EQAs and the budget to cover participation fees will be submitted to the Belgian National Institute for Health and Disability Insurance (RIZIV-INAMI) for evaluation and approval.

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P14.048C

Development and characterization of a sample to answer Factor II/V assay

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Introduction: Prothrombin G20210A and Factor V Leiden (G1691A) are dominant autosomal mutations that increase the risk of blood clots, including deep vein thrombosis and pulmonary embolism. Here we present the development and characterization of a Factor II/V real-time PCR genotyping assay compatible with Luminex's ARIES® System, a sample to answer device capable of running laboratory developed tests.

Materials and Methods: Fluorescently labeled modified primers were designed to bind to wildtype and mutant Factor II/V alleles with the 3' end of the primer located at position 1691 for Factor V and 20210 for Factor II. DNA extraction and real-time PCR were performed using the ARIES® System, ARIES® Extraction Cassettes, and DNA Ready Mix (an ARIES® compatible lyophilized master mix). Performance was evaluated using EDTA-stabilized human blood samples, banked nucleic acid with known genotypes, and synthetic DNA.

Results: All Factor II/V genotypes were correctly identified in a set of 98 fresh human blood samples and 43 previously characterized DNA samples. SNPs near the mutations showed no impact on assay performance. The sample to answer assay required only 5 minutes hands-on time and tolerated common blood interfering substances and variation in blood input volumes from 50 to 500 µL.

Conclusions: The Factor II/V assay presented here is both highly specific and robust. The assay's compatibility with the sample to answer ARIES® System significantly reduced the hands-on time required to perform the test.

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P14.049D

Improved fetal fraction estimation by combining estimators based on fragment lengths and fragment counts in non-invasive prenatal testing

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Introduction: The reliability of non-invasive prenatal testing is highly dependent on the accurate estimation of fetal fraction. Several methods have been proposed up to date, utilizing different attributes of analysed genomic material, for example length and genomic location of sequenced DNA fragments. These sources of information are relatively different, but so far, at least according to our knowledge, there were no published attempts to combine them to get an improved predictor.

Materials and Methods: We propose several fetal fraction estimation improvements with special focus on the samples that are most prone to wrong diagnostic conclusions. We introduced new method for estimating fetal fraction (Non-linear regression model) and also compared other statistical (Linear regression model) and machine learning methods (Neural networks, Support vector machines).

Results: We demonstrate, that although understudied, prediction based on length of sequenced DNA fragments may achieve nearly the precision as state-of-the-art methods based on their genomic locations. Finally, we show that combination of several sample attributes leads to a predictor that has superior prediction accuracy over any single approach.

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P14.050A

Performance of rapid DNA extraction from buccal swab using a new molecular system for the detection of a single nucleotide polymorphisms (SNPs)

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Introduction: SNP genotyping measures genetic variations of single nucleotide polymorphisms (SNPs). HPV infections immune-response have been implicated in cervical cancer and tumor necrosis factor-alpha (TNF-a) development associated to di SNP:rs 1800629. Bioside developed a rapid DNA extraction and lyophilizate ready-to-use SNPs systems. The objective of this study was to validate the Copan hDNA free FLOQSwab (hDNAFS) for buccal samples (BS) collection with the Bioside rapid DNA extraction and new SNPs systems.

Materials and Methods: In this study DNA quantity/quality of BS, collected with hDNAFS, cellulose, polyester and rayon swabs, were compared. BS (N=50) were used for this validation. Thermo Scientific (TF) SAMPLE TO SNP

system (ST-SNP-S) and Bioside DNA extraction system, (equipment-free buffer that extracts DNA in 10min RT incubation) analyzed with TF ST-SNP-S and the Bioside ready- to-use SNP-rs 1800629 analysis system for the detection of the TNF-A, A>G mutation. Genotyping was performed by sequencing and quantification by luminometer.

Results: Optimal DNA quantity/quality was obtained with hDNAFS BS and Bioside extraction. In the 50 BS analysed with both SNP systems, hDNA FS + Bioside obtained an average DNA better yield of 0.5 log compared to hDNAFS +TF ST-SNP-S. In the samples tested 50/50 were correctly genotyped by the Bioside-system and 49/50 by TF ST-SNP-S. Inhibition was detected in 2/50 by ST-SNP-S and in 0/50 by Bioside-system.

Conclusions: Better DNA yield and genotyping without-inhibition was obtained from BS collected with Copan hDNAFS by both Biooside rapid nucleic acid extaction and ready-to- use lyophilizate SNP-rs 1800629 analysis for TNF-A, A>G mutations.

B. Mombelli: None. **M. Boles Savoldi:** None. **S. Castriciano:** None.

P14.051B

CRISPR/Cas9-targeted enrichment and long-read sequencing of a non-coding corneal dystrophy-associated *TCF4* triplet repeat

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Introduction: More than 40 human diseases are caused by expansions of simple nucleotide repeat sequences (micro-satellites). Diagnosis and prognosis of these conditions relies upon accurate sizing of mutant alleles for which current PCR-based sizing methods are insufficient. Here we demonstrate the custom application of an amplification-free long-read sequencing method, termed 'No-Amp Targeted Sequencing', to characterise the Fuchs endothelial corneal dystrophy (FECD)-associated intronic TCF4 triplet repeat (termed CTG18.1) at the nucleotide level within a FECD patient cohort.

Materials and Methods: We applied an amplification-free method, utilising the CRISPR/Cas9 system, in combination with PacBio single molecule real time (SMRT) long-read sequencing, to study CTG18.1. FECD patient samples (n=11) displaying a diverse range of CTG18.1 allele lengths and zygosity status (as determined by conventional PCR-based methods) were analysed. A robust data analysis pipeline was developed to effectively filter, align and interrogate CTG18.1-specific reads.

Results: CRISPR-guided SMRT sequencing of CTG18.1 provided accurate genotyping information for all samples and phasing was possible for 18/22 alleles sequenced. Repeat length instability was observed for all expanded (≥50 repeats) phased CTG18.1 alleles analysed. Furthermore, higher levels of repeat instability were associated with increased CTG18.1 allele length (mode length ≥91 repeats) indicating that expanded alleles behave dynamically.

Conclusions: CRISPR-guided SMRT sequencing of CTG18.1 has revealed novel insights into CTG18.1 length instability. Furthermore, this study provides a framework to improve the molecular diagnostic accuracy for CTG18.1-mediated FECD, which we anticipate will become increasingly important as gene-directed therapies are developed for this common age-related and sight threatening disease.

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P14.052C

Challenges, warnings and recommendations for gene panel testing in hereditary cancer

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Since the incorporation of NGS into genetic diagnostics, the laboratories test-portfolio has broadened. However, the analysis of multiple genes at once is not always paired with enough knowledge regarding molecular pathology, genetic utility, associated risks and clinical management. Our aim is to discuss some of the current challenges in hereditary cancer testing.

Concerning special gene features:

-Not always truncating variants in tumour suppressor genes are pathogenic, due to functional rescue by other transcripts (i.e. *BRCA1*, *TSC2*).

-Several genes have nearly identical sequences and exist in multiple locations as a result of segmental duplications. This fact can cause an important decrease in the accuracy of variant detection, even reporting pseudogene variants as pathogenic. We face this problem in *PMS2*, *PRSS1*, *BMPR1A*, *CHEK2*, *BRCA1*, *NF1* and *PTEN*.

Concerning variant classification:

-Some hereditary cancer conditions are caused by activating variants, so the widely used ACMG/AMP classification guidelines should be applied with caution.

-In the same gene, different pathogenic variants can confer different cancer risk.

-Exon/gene duplications are not necessarily pathogenic. It is mandatory to prove in each case that the variant damages the transcript.

-ClinVar clinical assertions should not be directly transferred to a diagnostic report since some are based on insufficient evidence.

In the "one-gene-at-a time" era, finding a pathogenic variant often led to stop the analysis. However, comprehensive (sub)exomic analysis reveals numerous cases of digenic, trigenic, and more complex inheritance patterns as seen in the increasing number of patients with Multilocus Inherited Neoplasia Alleles Syndrome (MINAS).

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P14.053D

Optimizing diagnostic sequencing to both improve clinical sensitivity and reduce confirmatory testing: An interlaboratory approach

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Introduction: To reliably inform medical care, clinical sequencing must provide high sensitivity for pathogenic variants. This can be difficult to achieve given the high prevalence of technically challenging pathogenic variant types in patients[1]. Unfortunately, the highest sensitivity NGS methods can also produce false positives, necessitating the use of orthogonal confirmatory assays, a significant cost and time burden. Optimizing both sensitivity and specificity at minimal total cost may be aided by careful analysis of large clinical data sets.

Methods: Thirteen validated genetic tests in two clinical laboratories were applied to a suite of reference samples and over 50,000 patients with orthogonal data. A novel optimization algorithm divided these variants into classes based on NGS quality metrics, type and genomic context. Each class was characterized using rigorous statistics.

Results: Most pathogenic variants (90%) could be placed into classes having perfect observed accuracy (both sensitivity and analytic PPV) with CIs 99.5% or better. Strong claims regarding sensitivity for such variants would be justified, and these variants do not benefit from confirmation. The remaining variants require additional steps to detect (e.g. additional algorithms and low stringency filtering, to reduce false negatives) and often do require confirmation (to reduce false positives).

Conclusion: Our methods are applicable to panels, exomes, and genomes. They described in detail and can be applied by many laboratories[1,2]. These methods and data may inform emerging international guidelines on clinical NGS[3].

References: [1] Lincoln ESHG 2018; Manuscript in review; [2] Lincoln JMD in press; [3] Crooks AMP 2018; Manuscript in preparation

S. Lincoln: A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Illumina, Thermo Fisher. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Invitae. **M. Lebo:** None. **J. Zook:** None. **R. Truty:** A. Employment (full or part-time); Significant;

Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Invitae. **C. Lin:** None. **J. Paul:** A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Invitae. **V. Ramey:** A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Invitae. **M. Salit:** None. **H. Rehm:** None. **R. Nussbaum:** A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Invitae.

P14.054C
Patient blood derived cell lines provide unlimited supply of DNA

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Background: Advances in genomic profiling techniques have enabled researchers to identify risk factor genes that can contribute towards an individual's susceptibility to a disease. The validity and reproducibility of research relies on access to ethically sourced, authenticated, quality controlled samples. The UK MND Collections (formerly the UK MND DNA Bank) is a biological resource, containing DNA, cell lines and epidemiological data for MND research.

Objectives: To collect and make available samples, and associated clinical data, from cohorts of patients, their parents/siblings and controls, representing cases of sporadic and familial MND for research into genetic risk factors.

Methods: Over 3000 patient and control blood samples were collected and sent to the European Collection of Authenticated Cell Cultures for storage and Epstein-Barr virus (EBV) transformation into lymphoblastoid cell lines (LCL). EBV transformation had been demonstrated to be an efficient, cost-effective method for immortalising patient samples, allowing for a potentially unlimited supply of DNA. The LCL underwent authentication testing to ensure that they matched the original patient blood sample and the respective DNA.

Results: LCL were generated from all blood samples and in 2017, the MND Association selected 193 samples to be made available to researchers. Gene mutations present in this sub-set of cell lines include C9orf72, SOD1, FUS and TARDBP.

Discussion: To support MND research the patient and control cell lines are available from ECACC following approval from the MND Association. We would like to thank the MND Association, and the Wellcome Trust for funding the formation of the cell line collection.

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P14.055B
GenoinVar: an end-to-end solution to the discovery of causal variants

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Genetic diagnosis is crucial information for clinical practice in many cases. The untargeted solutions based on next-generation sequencing have proved to be cost-effective in the diagnosis of a wide range of conditions and have also led to a significative reduction in time to diagnosis.

We developed GenoinVar, an integrative end-to-end solution from the biological sample to exome sequencing and variant analysis to the discovery of causal genetic variants. The solution involves Illumina whole exome sequencing using a capture procedure from IDT, with uniform and above specifications sequencing metrics. Sequencing data is processed using an in-house pipeline, and an encrypted database of variants with the corresponding annotations is created. Candidate variants are prioritized in our ExomeLoupe platform, an intuitive and user-friendly Windows software for variant selection and interpretation. ExomeLoupe interacts directly with the encrypted database enabling users to securely store, analyze and share this sensitive genetic information in compliance with the GDPR.

This end-to-end solution was developed in the framework of In2Genome, a multidisciplinary project to integrate exome sequencing in clinical practice, with experts of Genoinseq, Coimbra Genomics and the Genetics Unit of the Coimbra Pediatric Hospital (CHUC). GenoinVar validation included 13 molecular diagnosed patients selected by clinical geneticists. The GenoinVar sequencing results were analyzed by two independent blinded specialists using ExomeLoupe that successfully identified the previously reported causal variants in all 13 cases.

GenoinVar represents a true end solution for identifying causal variants in clinical and research contexts.

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P14.056C

A new diagnostic approach to calling CNVs from low read-depth genome sequencing data

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Low read-depth genome sequencing (GS) has been demonstrated to detect CNV and proposed as a cost-effective, resilient alternative to cytogenomic microarrays (CMA). We aimed to assess this technology in comparison to the current standard of care.

GS data with an average read depth of 5X was processed with a number of CNV callers to evaluate performance. These included: WisecondorX, Canvas, Lumpy, Manta, Breakseq2, Breakdancer, Delly, and CNVnator. We also investigated a CNV caller developed by the Leeds MRC Medical Bioinformatics Centre. Data was downsampled in order to test these algorithms at a series of read depths. After optimising the algorithms by modifying the seed length or window size, we demonstrated detection of CNVs down to 0.3Mb at a read depth of 0.05X. To determine whether this method is a feasible and cost effective, samples with known CNV calls were prepared using the NEXTFELX Rapid DNA-Seq kit, sequenced on the Illumina NextSeq platform and analysed using the refined pipeline. To match the current cost of CMA analysis, each sample can be sequenced to roughly 0.225X. Furthermore, tissue samples were run to investigate the resilience of low read depth GS for poor quality samples. In conclusion, low read-depth GS performed similarly to CMA for CNVs >0.3Mb, even at read depths as low as 0.05X. Although issues surrounding mapping repetitive regions and breakpoint accuracy need to be resolved, we have demonstrated that low read-depth GS can offer an improved, low-cost service in comparison to CMA.

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P14.057D

Outcome of genomic testing in in South Australia: detection rate is not the same for all (virtual) panels

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Introduction: We audited our germline genomic testing in South Australia using massively parallel sequencing from 2014 to 2018.

Method: Data were extracted from laboratory records. Testing was initially performed on the Illumina Inherited Disease™, Trusight Cancer™ and Trusight One™ sequencing panels, dependent on genes requested. We currently use virtual panels on either a custom-designed Roche 1228 gene panel or the Roche NimbleGen SeqCap EZ MedExome. Libraries were sequenced on the Illumina MiSeq or NextSeq sequencing systems. Results were classified according to the ACMG standards. Pathogenic/likely pathogenic results were confirmed by Sanger sequencing.

Results: 3068 tests were performed. The highest diagnosis rate (98%) was for confirmation of a biochemical diagnosis. High diagnosis rates were achieved for Duchenne muscular dystrophy (71%, 15% VUS), Alport syndrome (55%, VUS 7%), neuromuscular (48%, VUS 38%) and connective tissue (42%, VUS 32%) panel testing. The lowest diagnostic rates were seen in Ehlers Danlos (9%, VUS 55%), Noonan (27%, VUS 20%), Cornelia de Lange (29%, VUS 43%), single gene/small panel (28%, VUS 21%) and large gene panel testing (22%, VUS 24%). Sanger sequencing confirmed 1460 of 1478 positive results (99%). 6 non-confirmations related to repeat regions, 4 each to sample mix up, pseudogenes and "noisy" regions.

Conclusions: We are now testing more exomes, with a preference for phenotype-driven analysis. As expected, genes for which molecular testing has been performed longer have more positive diagnoses and less VUS. There was a high concordance for variant confirmation.

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P14.058A

Advantages of genotype imputation with ethnically matched reference panel for rare variant association analyses

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Genome-wide association studies (GWASs) have been widely used for detecting genetic biomarkers in a wide range of traits. Typically, GWASs are carried out using chip-based genotyping data, which are then combined with a more densely genotyped reference panel to infer untyped genetic variants in chip-typed individuals. Publicly available and ethnically heterogeneous imputation reference panels (IRPs) such as 1000G are sufficiently accurate for imputation of common and low-frequency variants. Population-specific IRPs have demonstrated great improvement in imputation accuracy of rare variants, but their effect to the downstream analysis is not very well examined.

We systematically compared downstream association analysis effects in ~37,000 Estonians imputed with ethnically mixed 1000G and ethnically matched Estonian-Finnish IRPs. Firstly, variant-wise GWASs were performed on eight complex traits (body mass index and seven complex diseases of major public health importance). Although several previously reported common variant associations were replicated in both imputed datasets, no major differences were observed. Secondly, a gene-based tests of rare (MAF < 1%) nonsynonymous and loss-of-function variants were conducted and identified significant gene-trait associations were studied in the UK Biobank data. Gene-based analysis demonstrated that ethnically matched panel outperformed the 1000G-based imputation, provided 10-fold increase in tested genes and significant findings. Validation indicated that most of the significantly associated genes were previously known, but there were some which turned out to be worthwhile novel findings.

In conclusion, we observed that population-specific panel ensures a better imputation quality for rare variation and captures more population-specific variants, enabling more efficient discovery of disease-associated genes.

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P14.061D
Identification of a novel germline BRCA2 duplication by targeted gene enrichment and next generation sequencing

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Introduction: About 70% of women with a pathogenic germline variant in *BRCA1* or *BRCA2* (*BRCA1/2*) will develop breast cancer by the age of 80. The identification of *BRCA1/2* mutations is required to estimate the cancer risk for relatives, initiate preventive measures and determine the best choice of treatment. Although multiple screening techniques are routinely applied, genetic predispositions in *BRCA1/2* and other core risk genes are identified in approximately 40% of hereditary breast cancer cases.

Materials and Methods: By multiplex ligation-dependent probe amplification (MLPA) of *BRCA2*, a duplicated region spanning exon 22 to 24 was detected in a patient with bilateral breast cancer at age 35 and 44. To determine the location and orientation of the duplicated area, targeted enrichment of the genomic *BRCA2* locus followed by NGS was performed. Its spatial properties were identified by the bioinformatic tool CNV-Hunter and validated by breakpoint spanning PCR and Sanger Sequencing. Additionally, the probands' four sisters were tested for the duplication via Sanger Sequencing.

Results: Using NGS, we showed the tandem organization of the *BRCA2* exon 22 to 24 duplication, Chr13(GRCh37): g.32951528_32960522dup, in the proband, but not in her sisters. Theoretically, the duplication causes a frameshift and leads to a premature stop codon (p.Ala3088PhefsTer3). This may lead to nonsense-mediated mRNA decay and is likely to be pathogenic.

Conclusions: We demonstrate rapid and accurate identification of a genomic rearrangement using next generation sequencing. This case study demonstrates the ability of high-throughput sequencing to detect and characterize, besides single nucleotide variants, large genomic rearrangements.

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P14.062A
Copy number variant detection tool for targeted sequencing data

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Introduction: With the use of NGS genepanels in genetic diagnostics, more genes are now being tested compared to a few years ago. Copy Number Variant (CNV) detection is often done using MLPA, which is labor intensive, expensive and limited to some specific genes. As more genes are being tested, the amount of MLPA testing has become massive and unmanageable. Therefore, the Department of Medical Genetics at St. Olavs Hospital (Norway) has developed an in-house CNV detection tool for targeted sequencing data.

Materials and Methods: The "in-house" tool calculates CNV detection scores by comparing coverage depths of a test-sample with a pool of normal samples. A sliding-window approach is used for dynamic region selection. Validation of the tool included 36 positive controls with CNVs in 12 genes and 11 negative controls.

Results: All the CNVs of the positive controls were successfully detected resulting in a measured sensitivity of 100%. The specificity was measured to be 91% were most of the false positives were due to systematic errors in challenging genomic regions (e.g. high GC –content, low coverage, high homology).

Conclusion: Our CNV detection tool has been validated and established in routine diagnostics of hereditary cancer at our department. CNV detection using targeted NGS data makes it possible to broaden our genetic testing services to also include CNV detection of genes where there is no MLPA analysis available. This tool is shown to be sensitive and specific in addition to time- and cost-effective.

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P14.063B
Sample quality control of long read sequencing and low input libraries

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Long-read sequencing and miniaturization of library preparations are becoming increasingly common as new next-generation sequencing workflows are developed. Traditional quality control methods do not provide the required sizing accuracy of DNA greater than 50kb or the sensitivity allowing for sample conservation during the quality control assessment steps. The Femto Pulse system by Agilent Technologies works to streamline quality control by separating genomic DNA up to 165kb in as little as 70 minutes, down from the 16+ hours required for traditional agarose PFGE. The unparalleled single cell gDNA sensitivity of the Femto Pulse allows for preparation of low input NGS libraries from cfDNA, RNA, and miniaturized traditional DNA NGS libraries. Quality control metrics such as the RNA Quality Number (RQN) and user defined Genomic Quality Number (GQN) aids in the determination of sample quality/integrity. This poster shows the unique use of the Femto Pulse System in high molecular weight gDNA separation and low input library preparation with subsequent analysis features highlighted.

K. Luttgeharm: A. Employment (full or part-time); Significant; Agilent. **J. Molitor:** A. Employment (full or part-time); Significant; Agilent. **R. Nitsche:** A. Employment (full or part-time); Significant; Agilent.

P14.064C
NeatSeq-Flow: A Friendly High-Throughput Sequencing Workflow Platform for Local Use by Programmers and Non-Programmers Alike

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Genomic experiments involving High-throughput sequencing usually produce large amounts of data, whose analysis requires multi-step workflows. Creating and executing these workflows locally is often time consuming and error prone, especially when considering projects with hundreds or thousands of samples, with many steps and plenty of intermediate files, or when the same analysis has to be repeated with different combinations of programs and parameters.

The NeatSeq-Flow platform allows for modular bioinformatics workflow design and execution on a local computer or, preferably, computer cluster, using either a command-line interface or a fully functional graphical user interface (GUI). Analysis programs can be anything executable from the Linux command-line, thus enabling use of cutting-edge (public, commercial or in-house) tools for high-throughput sequencing data analysis. Creation and sharing of new workflows is easy and intuitive. Furthermore, NeatSeqFlow provides ready-to-use workflows for common bioinformatics analyses.

To execute a workflow, users only need to specify the order and parameters of analysis steps and the location of input file. Workflow execution is parallelized on the cluster, and progress can be tracked in real time. Workflow results are neatly organized in an intuitive directory structure. All

workflow components and their order of execution are stored in one file, which together with the shell scripts produced by NeatSeq-Flow comprise a complete documentation of the workflow and enable future execution of the exact same workflow or modifications thereof.

All these features make NeatSeq-Flow an easy-to-use workflow platform without compromising flexibility, reproducibility, transparency and efficiency. NeatSeq-Flow is free available at https://neatseq-flow.readthedocs.io

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P14.065D
Testing the use of Human Phenotype Ontology (HPO) terms for the identification of causal variants in clinical exomes

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Introduction: Clinical utility of exome sequencing has been well established during the last few years. In this context, HPO terms provides an important source of information that allows to correlate phenotypes, diseases and genes.

Methodology: Exome data from 188 patients were evaluated retrospectively. Primitively, these patients were analyzed using 7165 disease associated genes, that was considered as the gold standard. Performance of three different gene prioritization strategies was compared: candidate genes, genes associated to HPO terms provided by the physician and genes associated to prioritized HPO terms.

Results: Clinical exome allowed the diagnosis of 35% (66/188) of the patients. The candidate gene strategy was positive in 15% of the patients (29/188), while the strategy based on the selection of genes through the combination of HPOs reached a 29% diagnosis (54/188). Using HPOs terms, an average of 796 genes was evaluated. The selection of genes based on the combination of prioritized HPO terms, obtained a diagnostic rate of 25% (45/188). A detailed analysis of positive cases not identified under any of these prioritization strategies (12/66), showed that the use of less specific HPO terms would help detect the affected gene in 8/12 cases.

Conclusion: The use of HPO terms is a strategy that allows the diagnosis in a high percentage of cases, but its efficacy depends significantly on the selection and prioritization of this terms. The use of a genotype-phenotype strategy allows greater diagnostic rate since there may be cardinal symptoms not identified during the clinical evaluation of the patients.

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P14.066A
Phenotero: annotate as you write

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In clinical genetics, the Human Phenotype Ontology as well as disease ontologies are often used for deep phenotyping of patients and coding of clinical diagnoses. However, assigning ontology classes to patient descriptions is often disconnected from writing patient reports or manuscripts in word processing software. This additional workload and the requirement to install dedicated software may discourage usage of ontologies for parts of the target audience.

Here we present Phenotero, a freely available and simple solution to annotate patient phenotypes and diseases at the time of writing clinical reports or manuscripts. We adopt Zotero, a citation management software to create a tool which allows to reference classes from ontologies within text at the time of writing. We expect this approach to decrease the additional workload to a minimum while ensuring high quality associations with ontology classes. Standardised collection of phenotypic information at the time of describing the patient allows for streamlining the clinic workflow and efficient data entry. It will subsequently promote clinical and molecular diagnosis with the ultimate goal of better understanding genetic diseases. Thus, we hope that Phenotero eases the usage of ontologies and controlled vocabularies in the field of clinical genetics.

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P14.067B
Inborn Errors of Metabolism (IEM) - analytical validation of NGS assays for confirmative diagnostics

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Introduction: MS/MS is routinely used for newborn screening nevertheless, it requires fast accurate and inexpensive confirmatory method due to high rate of false-positive results. We aimed to develop and validate 5 NGS assays for several IEM groups: organic aciduria (AO), fatty acid oxidation defects (FAO), aminoacidopathy (AA), vitamin B defects (AAVB) and carbohydrate disorders (GH). Material and methods: 5 IEM panels (4-37 genes, 15-158kbp) were designed for multiplex PCR targeted enrichment and evaluated *in-silico* before production. *In-vitro* assessment was performed on NIST RM-8398, 1000G reference materials and dried blood spot samples. Libraries were prepared with Parseq protocol based on targeted enrichment followed by adapter ligation, barcoding and sequencing on MiSeq and IonTorrent platforms. Alignment and variant calling were performed using VariFind™ Software. Analytical characteristics were calculated within all targeted regions (all variants and wild-type positions). Clinically relevant variants from ClinVar database were evaluated.

Results: Analytical performance of 5 IEM panels were established: sensitivity - 95.5-100%, specificity - 97.0-99.4%. Assays limitations and clinically relevant variants within regions with low quality were described.

Conclusions: 5 assays for confirmative IEM diagnostics are validated on Parseq protocol and pipeline, using MiSeq and IonTorrent (data not shown) platforms. IEM panels could be mixed in a single run. Assays are suitable for dried blood spot samples. Analytical performance of assays is sufficient for the further clinical trials. Diagnostic performance of assays as a second-tier test following MS/MS newborn screening is studied at the St.Petersburg's Medical Genetic Diagnostics Centre (Russia) and Medical Genetics Laboratory, Policlinico, Milan (Italy).

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P14.068C

The power of 16kb Long-Read Circular Consensus Sequencing for the homologous *OPN1* genes

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Pathogenic variants in the red and green cone opsins, encoded by *OPN1LW* and *OPN1MW* respectively, lead to visual impairment. The genes are located in tandem on Xq28 and show 98% homology. Current routine diagnostics uses gene-specific long-range (LR-)PCRs, followed by shearing and short-read sequencing, combined with MLPA, which are hampered by the high homology, variable copy numbers and occurrence of hybrid *OPN1* genes. We therefore investigated the power of long-read sequencing compared to standard short-read sequencing. Hereto, *OPN1LW* and *OPN1MW* of 47 known clinical samples were amplified using gene-specific LR-PCRs of ~16kb, and sequenced as 2x 8kb fragments. In addition, a subset was also sequenced as 16kb fragments. Library preparation, sequencing, and circular consensus sequence (CCS) analysis were performed according to PacBio protocols using the Sequel system.

All pathogenic variants and hybrid genes were confirmed using the 8kb read length, with the advantage of decreased hands-on time for data analysis and interpretation. Mapping of the 8kb fragments was however still hampered by high sequence homology. The latter was overcome by 16kb fragment long-read sequencing data, which showed improved quality and mapping compared to the 8kb reads. We therefore conclude that long-read sequencing can detect both point mutations and hybrid *OPN1* genes. With 8kb reads having the power to accelerate *OPN1* data analysis and interpretation in comparison to standard short-read sequencing, we expect that with further improvements using 16kb CCS reads, it will become possible to also detect *OPN1* copy number variants and point mutations using a single test.

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P14.069D

Molecular diagnostic challenges of repeat expansion disorders in the era of genome scale DNA sequencing: the myotonic dystrophy example

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Introduction: In general, molecular testing of repeat-expansion-disorders (REDs) require two complementing methods, while sequencing has no special place among them. Because of this, REDs patients yet have not benefited from the advent of massively parallel sequencing (MPS), a method that otherwise revolutionised differential diagnostics of the majority of monogenic and complex disorders.

Materials and Methods: We developed an alignment-free bioinformatics tool to characterize clinically relevant attributes of STR loci from MPS data sets. It was tested on a commercial targeted MPS panel, using different disease-associated STR loci. Validation was extended to samples having expansions in the myotonic dystrophy type 1 (DM1) associated *DMPK* gene. Results were validated by gold standard methods such as conventional and repeat-primed PCR. We then characterized aspects of MPS based REDs testing in routine practice.

Results: We were able to reliably genotype amplifiable STR alleles, determine their sequence structure, to phase individual alleles, and also to identify the presence of expanded alleles exceeding the amplification limit of the used MPS assay, overcoming thus the conventional amplification derived limitations of MPS. When compared to using MPS for more common variation types, such as single nucleotide variants, we found several practical problems, especially connected to automated annotation of identified repeat alleles.

Conclusions: Beyond technological questions, to fully benefit from MPS based differential diagnostics there are several aspects of testing which requires modifications when compared to current practice, from nomenclature of alleles, through database feeding, up to automated annotation of alleles. Supported by: APVV_-17-0526, VaV_MZSR_2018/46-SAV-5 and VEGA_ 1/0433/19.

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P14.070A
Consecutive medical exome analysis at a tertiary center: Diagnostic and health-economic outcomes

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Background: The utility of whole exome analysis has been extensively demonstrated in research settings, but its clinical utility as a first-tier genetic test has not been well documented from diagnostic and health economic standpoints in real-life clinical settings.

Methods: We performed medical exome analyses focusing on a clinically interpretable portion of the genome (4813 genes) as a first-tier genetic test for 360 consecutive patients visiting a genetics clinic at a tertiary children's hospital in Japan, over a 3-year period. Bioinformatics analyses were conducted using standard software including BWA, GATK, and SnpEff.

Results: A molecular diagnosis was made in 171 patients involving a total of 107 causative genes. Among these 107 causative genes, 57 genes were classified as genes with potential organ-specific interventions and management strategies. Hence, clinically relevant results were obtained in approximately a quarter of the patients. A cost-effectiveness analysis indicated that the overall cost would have been less expensive by 330 euros if a medical exome analysis had been performed at the time of the initial visit to a tertiary center, rather than after multiple visits to various specialists, a brain MRI examination, and G-banded chromosome analysis.

Conclusions: The present study demonstrated a high diagnostic yield (47.5%) for singleton medical exome analysis as a first-tier test in a real-life setting. From a public health policy standpoint, genomic testing can be more efficient for diagnosis with a cost that is comparable or less to that of currently used tests with lower diagnostic potential, including brain MRI and chromosome testing.

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P14.071B
Detection of IL-1 family rare genetic variants in metabolic disorders using single-molecule Molecular Inversion Probes (smMIPs)

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Introduction: The role of rare genetic variants has been neglected in obesity-associated metabolic disorders. In this study we investigated whether rare genetic variation in Interleukin-1 (IL-1) cytokine family members influences the development of obesity-associated metabolic disorders,

using an exon re-sequencing technology called single-molecule Molecular Inversion Probes (smMIPs).

Material and methods: We developed a unique MIP-panel for 48 genes encoding proteins involved in pathways related to IL-1 processing or -signaling. All coding exons were sequenced by smMIPs in two well-characterized Dutch cohorts of in total 703 obese patients with different metabolic complications (a.o. cardiovascular diseases, type 2 diabetes, and liver steatosis) and in a control cohort of 520 healthy individuals.

Results: Rare variants were defined as those with a minor allele frequency less than 1% in the exome-sequencing Genome Aggregation Database. In the obese cohorts ±62-68% of the total identified variants were rare, compared to ±73% in the healthy individuals. Among these rare variants 10-20% were newly described and 5-10% had a predicted deleterious effect on protein structure. In the obese patient cohort we observed an enrichment of rare variants in *ATG7*, *IL33* and *CASP1* as compared to healthy individuals; suggesting that rare variants in these genes may play an important role in metabolic diseases.

Conclusion: Here we show that smMIPs are a valuable technology for identifying rare variants possibly involved in metabolic diseases. This is a first step towards a better understanding of the role of rare genetic variation in metabolic disease.

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P14.072C
MethylCal: Bayesian calibration of methylation levels

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Background: The identification of clinically relevant epigenetic biomarkers and the translation to clinical diagnostics require reliable and accurate methylation assays. Targeted bisulfite sequencing has become the primary choice for single-base methylation quantification of multiple targets in parallel. Efficient bisulfite conversion is a critical aspect of this technique and incomplete conversion or the preferential amplification of an allele and strand can lead to inaccurate results. To avoid the cost and time of redesigning new assays, calibration methods based on standard controls have been suggested to correct PCR bias.

Methods: We have developed a novel Bayesian calibration tool, MethylCal, which analyses jointly all CpGs within a CpG island or a differentially methylated region (DMR) avoiding "one-at-a-time" CpG calibration.

Results: Compared to existing methods, MethylCal is more flexible and fitted the data better and provided a more accurate prediction of the methylation levels. We successfully tested MethylCal on six imprinting DMRs and two CpG islands and benefits of the new method, including the ability to detect outliers and impute missing values.

Conclusion: MethylCal with the specification of different random effects adequately accounts for the patterns of variances and correlations of the methylation levels across actual methylation percentages better than existing calibration tools.

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P14.073D
Effective faeces and vaginal, cervical, rectal and ear swabs preparation for metagenomic analyzes

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Background: Genetic identification of different elements of microbiome (bacteria, archaea, fungi, small eukaryotes, viruses) supports innovative personalized patients' treatment. Metagenome analyzes enable precise assessment of

both microbial community composition and its function. As biological material's variability (including physical features and microbiota abundance), the storage and experimental conditions cause biases, reliable and validated protocols are required. Aim: Establishing the microbiota genome extraction protocols and procedures toward metagenomics applications.

Materials and Methods: Newborns' faeces and various swabs types (auricle, vagina, cervix, rectum) stored at −80° C, were proceeded using different kits for microbiota extraction. The 16S rRNA sequencing has been performed using Illumina's MiSeq.

Results: We established microbiota extraction protocols' based on the same consumables for all types of samples, which enables adequate comparison analysis. The following difficulties were recognized: extremely low amount of biological material (swabs from newborns' auricle and faeces), mechanical lysis, presence of reaction inhibitors, host genome contamination, false results of samples concentration including aspects of DNA input's quantification for PCR reactions, and chimeras. Various extraction protocols' modifications were implemented: concentrating samples, applying selective primers and high-fidelity polymerase and carefully chosen numbers of PCR cycles. To verify assumptions 16S rRNA sequencing was performed and satisfying number of reads, % Reads Passing Quality Filtering, % Reads PF Classified to Genus and species diversity in various biological materials were received.

Conclusion: The optimized protocols ensure appropriate quality and quantity of material for downstream and high-throughput application in metagenomic studies. Support: Polish Diabetes Association 2015, 2017 and PUMS Medical Faculty' Scientific Grant 502-01-01110142-05618.

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P14.074A
Detection of MSI in circulating cell-free DNA from colorectal cancer patients

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Introduction: The success rate of check point inhibitors in the treatment of patients with colorectal cancers displaying microsatellite instability (MSI) underscores the need for novel techniques, because of the fact that serial tumor specimens are usually not available. In our study, we evaluated the liquid biopsy as an important source of cancer derived DNA for MSI detection.

Materials and Methods: Formalin-fixed paraffin-embedded (FFPE) tumor tissue and liquid biopsy samples were collected from 23 colorectal cancer patients. Genomic DNA and circulating cell-free DNA (ccfDNA) were isolated from blood samples, then concentrations and qualities were compared.

Testing for MSI was performed using kits obtained from Promega Corporation. Microsatellite alterations were scored by comparing the electrophoretic profiles of each biomarker (targeted gene regions of BAT-25, BAT-26, NR-21, NR-24, MONO-27, Penta-C and Penta-D) in genomic DNA versus ccfDNA.

Results: Of the 23 patients, the electrophoretic profiles of microsatellite biomarkers tested in ccfDNA matched those in the respective primary tumors in all cases with the higher quality in liquid biopsy samples relatively.

Conclusions: There was an excellent concordance between liquid biopsy samples and FFPE tumor samples to detect MSI. Moreover, liquid biopsy might be useful in colorectal patients undergoing treatment, that can be readily obtained using minimally invasive procedure.

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P14.075B
Nanopore sequencing via targeted enrichment for medical diagnostics

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Introduction: With nanopore sequencing rapidly evolving, it is now possible to routinely sequence individual DNA strands in the kilobase range, independent of their base composition. However several aspects, like lower base accuracy and relatively high whole genome sequencing costs have hindered nanopore sequencing from becoming established in medical diagnostics. Here, we describe our targeted approach for the analysis of patient samples using the MinION sequencing device (Oxford Nanopore Technologies, ONT), addressing several types of variants relevant in human genetics.

Materials and Methods: Long-range PCR was performed for target enrichment, with high molecular weight DNA or cDNA as input. Library preparation was performed using the SQK-LSK108 sequencing kit (ONT) and either R9.4 or R9.5 flow cells were used for sequencing on a MinION device. Base calling was carried out using Albacore and BWA mem or minimap2 were used for alignment.

Results: A 2h MinION run on a long-range *ACVRL1* product (cDNA) yielded over 300,000x coverage, with 50% of the reads showing the aberrant transcript. Even the reuse of flow cells yielded sufficient reads for the identification of the genomic breakpoints of a large *EPCAM* deletion and allowed the phasing of two *NPC1* variants (one of them *de novo*) revealing that both variants are *in trans*.

Conclusion: Nanopore sequencing combined with long-range PCR is a cost effective and rapid tool to address complex variants. Traditional PCR limitations, such as loss of base modifications, enrichment of repeat expansions or large regions (>20 kb), could be solved by application of CRISPR-Cas enrichment techniques.

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P14.077D
Effective mitochondrial genome reconstruction from total RNA sequencing

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Introduction: Variants in mitochondrial genome have an impact on several inherited diseases as well as they may trigger the formation of a tumor or influence the behaviour of cancer. However, the analysis of mitochondrial DNA (mtDNA) and detection of mutations is problematic due to a low portion of mtDNA in total cellular DNA and phenomenon of heteroplasmy that occurs in mitochondria. Considering human mtDNA does not contain introns, the reconstruction of whole mitochondrial genome through RNA sequencing seems to be effective.

Materials and Methods: VERO E6 lineage of cells was used as a material for molecular analysis. RNA isolation was accomplished from the cell suspension and subsequent reverse transcription were followed by preparation of DNA library. DNA analysis processing was automated using pipelines implemented in the SnakeLines framework running on the Snakemake workflow engine.

Results: The data from sequencer were processed by de novo assembly method to generate contigs from short fragments without the aid of reference genome. We were able to reconstruct total mtDNA by RNA sequencing where the most problematic were RNA non-coding regions, particularly the D-loop region. However, using our method, we were able to sequence also non-coding regions.

Conclusion: mtDNA represents a small but significant segment of the total DNA of the individual. The very small amount compared to genomic DNA is the enormous problem in its successful study. Therefore, our method is not based on direct DNA sequencing for mitochondrial genome reconstruction, but on RNA sequencing of cell lysate.

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P14.078A
Evaluation of two molecular methods to detect CALR mutations in myeloproliferative neoplasms

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Background: Myeloproliferative neoplasms (MPN) are hematopoietic disorders characterized by abnormal proliferation of the myeloid lineage. Three classic subtypes are polycythemia vera (PV). Essential thrombocythemia (ET) and primary myelofibrosis (PMF). These disorders are well known for their association with the JAK2 V617F mutation, in addition to mutations on MPL exon10, and JAK2 exon 12. CALR mutations were detected in approximately 20% to 25% of patients with ET and PMF and not in patients with PV. Most CALR mutations were deletions and insertions in exon 9, which cause frameshift mutations.

Methods: This study included 60 patients with MPN in Taiwanese population. We identified CALR mutation in patients with MPN by high-resolution melting (HRM) analysis. Additionally, HRM analysis was compared with ipsogen CALR RGQ PCR. To confirm the results of HRM and ipsogen CALR RGQ PCR, sequencing analysis was also carried out for all samples.

Results: Up to 6.25% of the CALR mutation was successfully detected in patients with MPN using HRM

analysis. Four out of 60 patients (6.67%) were positive for the presence of CALR mutation including p.L367fs*46 and p.K385fs*47. The results proved 100% comparable to those obtained by ipsogen CALR RGQ PCR.

Conclusions: The HRM analysis and ipsogen CALR RGQ PCR are feasible and reliable technique for the detection of CALR mutation. Furthermore, the HRM offers several benefits including saving of time, non-expensive, fast and lower workforce.

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P14.079B
Nanoliter-scale automation of library prep solutions for RNA sequencing

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RNA sequencing (RNA-seq) provides a more precise measurement of transcript levels and their isoforms than other expression profiling methods. We have developed an elegant microfluidics-based workflow and chemistry for RNA-seq library prep (LP). This method performs bead-based sample capture and elutes polyadenylated RNA from total RNA samples. The workflow supports reverse transcription, adapter addition and sample indexing PCR. The entire workflow is automated within a compact nanoscale integrated fluidic circuit (IFC) in a microtiter plate format. The workflow supports simultaneous processing of up to 48 samples. We developed two LP methodologies that use similar chemistry and workflow, one for the 3′-end of transcripts and the other for full-length transcripts enabling detection of alternatively spliced isoforms.

To demonstrate performance, we processed samples containing 10 ng and 100 ng of UHRR RNA + ERCC Mix 1 and Human Brain RNA + ERCC Mix 2. We loaded samples and sample barcodes into their corresponding inlets along with reagents on the IFC. After a single button push to initiate the workflow, the bead affinity column was formed, polyA RNA were enriched and template RNA was converted into cDNA, which was amplified with sample indexes. Finally, we pooled, purified, quantified and sequenced harvested libraries on an Illumina® NextSeq™ system.

Results show that the performance of this workflow surpasses conventional methods in the diversity of detected transcripts across the full range of input amounts. Our IFC technology automates the RNA-seq library prep workflow and reduces reagent use while improving performance.

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P14.080C
The development of a fast newborn screening method for common neonatal metabolic disorders based on nanopore sequencing technology

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Introduction: Inherited metabolic diseases (IEM) are usually devastating, and caused by single gene defects. In newborns, for the differential diagnosis of IEM, a number of laboratory tests must be performed. During these intensive investigations, a vast majority of severely ill patients die without a definite diagnosis. Recently, a number of newborn screening tests based on next-generation sequencing (NGS) have been develop to replace expensive and time consuming conventional laboratory tests. In this study, we describe a rapid and effective screening test for 3 severe metabolic diseases (maple syrup urine disease, biotinidase deficiency and mucopolysaccharidosis type-1) based on Oxford Nanopore Technology (ONT).

Materials and Methods: PCR based target enrichment was performed on all exons of the 5 genes (*DBT, BCKDHB, BCKDHA, IDUA, BTD*) responsible for the 3 diseases. Multiplex PCR studies were performed using super-fast

thermal cyclers and fast polymerases. The amplified regions were then sequenced with the ONT MinION device. Results were verified with the Illumina Miseq platform. Based on the data obtained, the appropriate bioinformatic tools were selected and their parameters were optimized.

Results: Using ONT, satisfactory molecular genetic screening results were obtained for in a far shorter time period (below 4 hours), than is currently available utilizing NGS. Despite the protocol being highly successful for single nucleotide variations, for insertions and deletions artifacts were present.

Conclusions: This is the first study developed using ONT for the screening of metabolic disease. The development of specific bioinformatic tools is necessary to further improve the speed and accuracy of the results.

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P14.081D

Medical costs of children admitted to the Neonatal Intensive Care Unit; the role and possible value of rapid Whole Exome Sequencing

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Introduction: Novel genetic technologies are promising but often come at high costs. To adequately quantify the impact of their introduction in patient care, it is essential to have detailed insight in current costs of diagnostic procedures. We provide an overview of average healthcare costs for patients admitted to the Neonatal Intensive Care Unit (NICU) to facilitate discussion on the possible impact of rapid Whole Exome Sequencing (rWES) on total healthcare costs.

Methods: We retrospectively collected postnatal healthcare data of 1,423 children admitted to the NICU at the Radboudumc between October 2013 and October 2015. We distinguished between children with a conclusive molecular diagnosis found within the first 30 days of life (for comparison to rWES) and those genetically diagnosed after this period.

Results: Overall, the average costs spent were €24,357 per patient. Of the 1,423 patients, 149 (10.5%) underwent genetic diagnostic testing. In 13 (8.7%) a genetic diagnosis was obtained during the first 30 days of life and for 35 (22.8%) thereafter. For patients with a genetic diagnosis <31 days of life, total costs spent on genetic diagnostics accounted for 3.4% of all costs (€1.842 per patient), whereas for those receiving a genetic diagnosis >30 days, costs for genetic testing were 5.0% (€3.495 per patient) of total costs.

Conclusions: Genetic diagnostic testing in a NICU patient cohort accounts for a small fraction of total costs. With an anticipated increase in genetic diagnoses, a shorter time-to-diagnosis, and reduction of sequencing costs, implementation of rWES for this patient cohort may be warranted.

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Phenotypic characterization and functional analysis of cases suspect for Neurofibromatosis type 1

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Background: Neurofibromatosis type 1 (NF1) mutation detection and variant interpretation is challenging. We compared clinical characteristics of patients with NF1-variants of unknown significance (VUS) or no mutation identified (NF1-NMI) to those with pathogenic NF1 variants. Secondly, we performed *in vitro* functional assessment to reclassify NF1 VUS.

Methods: *Clinical characterization* Clinical and genetic information of clinically suspected NF1 patients was extracted from the departmental NF1 database (1993-2016). Characteristics of the NF1-VUS and NF1-NMI groups were compared to the NF1 pathogenic variant group. *Functional characterization of NF1-VUS* Expression constructs encoding *NF1* VUS were derived by site-directed

mutagenesis. By in vitro assays, we estimated the RAS GAP activity of the neurofibromin variant proteins using a RAS-GTP pull-down assay. Secondly, we used coimmunoprecipitation to investigate whether the interaction between neurofibromin and SPRED1 was affected.

Results: We identified 417 (68%) pathogenic *NF1* variants, 33 (5%) VUS and 167 (27%) NF1-NMI individuals. The percentage of patients in each group who met the clinical NF1-NIH criteria was 64%, 67% and 34% respectively. In the NF1-NMI group 45% of the individuals only had one NF1-NIH criterion identified. Functional assessment on 27 NF1-VUS resulted in reclassification of 6 VUS as pathogenic and 10 as likely pathogenic.

Conclusions: Clinically, the NF1-VUS group was largely comparable to the pathogenic variant group. Functional analyses were useful to further classify *NF1* VUS. Due to the clinical and familial consequences of an NF1 diagnosis, future studies should focus on optimizing assays of neurofibromin function to facilitate establishing an accurate molecular diagnosis.

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Targeted next generation sequencing for genetic testing of inherited diseases with NEBNext Direct

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Next generation sequencing is increasingly being adopted for the screening of inherited disorders. A patient's genetic information is useful for the diagnosis, prognosis, and management of disease as well as factors impacting expanded familial screening. To demonstrate the applicability of NEBNext Direct target enrichment for genetic screening of germline variants, we have developed target-enrichment panels specific to inherited disease. Spanning from a single-gene panel for cystic fibrosis screening to a comprehensive panel for screening an array of cardiac-related diseases, the NEBNext Direct technology can be applied to a wide range of genomic targets, using a novel hybridization-based, single-day workflow. The approach rapidly hybridizes both strands of genomic DNA to biotinylated baits, captures the targets on streptavidin beads, enzymatically removes off-target sequence, and directly converts captured molecules into Illumina-ready libraries. The sequencing reads have highly defined start sites and produce uniform coverage across a given target. The NEBNext Direct CFTR Panel consists of a single pool of baits targeting both strands of DNA across 27 exons and 7 intronic sites, for a total captured territory of 6.751 kilobases. The panel enables screening and detection of over the 2,000 variants that have been recorded worldwide. The NEBNext Direct Cardiogenomics Panel covers 160 genes associated with a range of cardiomyopathies as well as Brugada, Noonan, Marfan, and Long QT syndromes. Here, we will demonstrate the utility of these panels for producing highly specific, highly uniform enrichment of gene targets prior to next-generation sequencing.

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P14.084C

Comparison of molecular inversion probe versus Truseq® custom methods for targeted next-generation sequencing in peripheral neuropathy

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Introduction: Resolving the genetic architecture of painful neuropathy, a disabling feature of peripheral neuropathy (PN), will lead to better disease management. Several targeted Next-generation sequencing (NGS) approaches are currently available. Aim of this study was to develop a reliable technique to rapidly and accurately re-sequence multiple genes in a large cohort of PN patients at a low cost.

Materials and Methods: We compared the sensitivity, specificity, efficiency, reproducibility and cost-effectiveness of TruSeq® Custom Amplicon (TSCA, Illumina) and Molecular Inversion Probes (MIPs) NGS methods. To target nine sodium channel genes, capture probes were designed using their respective informatics pipelines. One-hundred-sixty-six patients with PN were tested by both methods, 70 samples were also validated by Sanger sequencing.

Results: Approximately 39kb were sequenced. 95% of the targeted regions showed an average coverage of ≥20x in TSCA, and 96% in MIPs. Sanger sequencing showed a 100% agreement with MIPs and TSCA. Sensitivity, specificity, performance of the two techniques were comparable, showing user-*friendly software to design probes and* similar on-target efficiency. MIPs has a more versatile assay design, flexible, allowing probes re-placement and integration. TSCA results less flexible, but probes dosage adjustment was unnecessary. The per-sample price for MIP was about 10x less than TSCA.

Conclusions: MIPs approach is more suitable for wide NGS panels on high sample numbers, reducing single-sample pricing if processed on a high-performance platform, whereas TSCA seems to be very time-effective and could represent the best solution to analyze smaller sample sizes on a cheaper flow-cell. (FP7-PROPANE Grant n.602273)

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P14.086A

Innovative method for reducing uninformative calls in non-invasive prenatal testing

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Introduction: Non-invasive prenatal testing or NIPT is currently among the top researched topic in obstetric care. While the performance of the current state-of-the-art NIPT solutions achieve high sensitivity and specificity, they still struggle with a considerable number of samples that cannot be concluded with certainty. Such uninformative results are often subject to repeated blood sampling and re-analysis, usually after two weeks, and this period may cause a stress to the future mothers as well as increase the overall cost of the test.

Materials and Methods: We propose a supplementary method to traditional z-scores to reduce the number of such uninformative calls. The method is based on a novel analysis of the length profile of circulating cell free DNA which compares the change in such profiles when random-based and length-based elimination of some fragments is performed.

Results: Although the proposed method is not as accurate as the standard z-score, the combination of these two independent methods correctly resolves a substantial portion of healthy samples with an uninformative result. Also the method can be used to identify maternal aberrations, thus reducing the risk of false positive and false negative calls.

Conclusions: Reliability of traditional NIPT tests based on low coverage sequencing can be improved by closer analysis of lengths of sequenced DNA fragments.

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P14.087B
Simultaneous detection of CNVs and SNVs by Oneseq technique: a retrospective study of 21 cases

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Introduction: Congenital malformations are found in approximately 3% of newborns. While improvement of fetal imaging allows their detection during pregnancy, genetic investigations carried out in the prenatal setting are most often limited to search for a genomic imbalance by karyotyping and/or chromosomal microarray analysis (CMA). Presently, molecular genetic analysis is rarely proposed due to the time and the cost needed to sequence a gene by Sanger, and the genetic heterogeneity of many syndromes. The objective of our study is to test a combined approach detecting Copy Number Variants (CNVs) and Single Nucleotide Variants (SNVs) in one experiment.

Materials and Methods: In partnership with Agilent, we adapted OneSeq® product for the prenatal diagnosis by selecting panels of genes involved in developmental disorders and using a similar strategy to our "custom chip" for the detection of CNVs. Validation was performed on a series of 21 fetuses or patients with known CNVs and/or SNVs and compared to the results previously determined by CMA and panels of genes.

Results: All the 10 CNVs larger than 500kb were identified by Oneseq® technique. Regarding the SNVs, they were all detected except one intragenic duplication in the *LBMR1* gene.

Conclusions: Our study demonstrates the reliability of the OneSeq® technique and emphasizes the advantage of having a simultaneous detection of CNVs and SNVs in the prenatal setting. Thus, a more precise genetic counseling can be provided to couples which can help them to make a decision on a possible termination of a pregnancy.

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P14.088C
Gene expression profiling of peripheral blood treated with different pretreatments and storage conditions

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Introduction: Peripheral blood, as a type of liquid biopsy, provides a source of non-invasive biomarkers for evaluation of health and disease, and RNA-seq is a powerful method to investigate genome-wide gene expression profiles. Various factors, for example, pretreatments and storage conditions of blood, which are critical in sample collection, could affect gene expression detection. However, how these factors would actually influence transcriptome remains largely unknown.

Methods: We used RNA-seq to explore the differences of expression profiles of samples from four pretreatments including Whole Blood (WB), Buffy Coat (BC), White Blood Cell (WBC) and Peripheral Blood Mononuclear Cell (PBMC) and the effects of storage conditions.

Results: Transcriptome of BC shows comparable globin mRNA ratio and gene detection ability with that of WB, while lower globin mRNA ratio and higher expressed gene number are observed in WBC and PBMC transcriptomes. Granulocytes related genes are well characterized in WBC. Transcriptome changes along with blood stored at both room temperature (RT) and 4°C, and more genes changes at RT especially genes related to granulocytes, while much fewer genes changed at 4°C. Reference genes such as CHMP2A and PSMB4 are relatively stable, while the expression of IL1RN, which is similar in both men and women, increases more in women than men after 24 hours stored at RT.

Conclusion: Our study provides a full-scale *ex vivo* change of peripheral blood expression profile, and suggests rigorous sample collection strategies to obtain authentic transcriptome.

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P14.089D
Phasing of *de novo* mutations using multiple amplicon long read sequencing

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De novo mutations (DNMs) play an important role in severe genetic diseases with an impact on fitness. Our research focuses on the role of DNMs in severe male infertility by studying unique cohorts of infertile patients and their parents. To better understand DNMs and their role in disease, it is important to determine the parent of origin and timing of DNMs. This in turn requires phasing of DNMs, which is usually impossible from short-read sequencing data as informative SNPs (iSNP) are often located 100-1000s of bases from a DNM. Therefore, we developed and validated a low-cost long-read sequencing approach. First, we identified all DNMs present in exomes from 108 patients with severe male infertility and their parents, using the Illumina Novaseq6000. Next, the short-read sequencing data was used to collect the location and genotypes of all iSNPs surrounding the DNMs. These target regions were then sequenced using the Oxford-Nanopore MinION. Several difficulties were addressed; DNA origin, amplicon size, coverage, and high basecalling error. Depending on amplicon read length and based on coverage obtained thus far, this approach can accurately phase ~100 *de novo* mutations per MinION run. This analysis indicated that 68% of the DNMs identified in these infertile men occurred in the paternal gamete pre-fertilization, not unlike what is known for DNMs in the normal population. From further analysis we identified the timing of all DNMs, noting 26% occurred postzygotically. Had these postzygotic DNMs occurred earlier they could have negatively impacted gametogenesis and/or the reproductive success of the parents.

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P14.090A
Feasibility of amino-acid sensing on dried blood spot using near infrared spectroscopy

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Introduction: We are developing a new, fast, non-destructive method to sense phenylalanine in human blood using Near Infra-Red (NIR) spectroscopy.

Methods: By using a sensor calibrated in the Near Infrared spectrum (optical window: 700nm to 1000nm), we acquired spectra from DBS card alone, DBS card with a reference concentration scale for Phe alone, and Phe plus Tyr.

Based on these data we selected the most sensitive wavelength window within the NIR and exploited this parameter to acquire spectra from DBS containing μ blood spiked with 0 to 1200 μM Phe, 0 to 1200 Tyr and with different Phe/Tyr ratios.

An additional experiment using 100 calibrators with 5 data points for phenylalaninemia was performed. For each sample, at least 20 scans in duplicate were acquired. Principal component analysis (PCA) was conducted.

Results: The analysis of the "scrubbed" data both by a specific spectrometry software and by data point algorithms, clearly show that it is possible to correlate the nature of the analyte and its relative concentration.

Acquired spectra present significative differences, and it is possible to isolate a specific Phe NIR fingerprint.

These observations open the exciting possibility to design a chemometric model to assay the amount of Phe in DBS

without elution and the expensive chromatographic procedures.

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P14.091B
Rapid clinical WES, 3 years of experience - challenges and choices

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The high diagnostic yield of clinical whole exome sequencing (cWES) has made this test the first choice for patients with (heterogeneous) genetic diseases. However, turnaround times are traditionally too long to serve patients who are in great need of a quick diagnosis. The demand for in house rapid cWES procedure increased swiftly from 1-2 trio samples a month in 2016 to 5-7 trio's per week in 2018. We are continuously optimizing our procedures and workflow to increase capacity, to better fit demands of patients and referring clinicians, but also to improve data analyses and diagnostic yield. This resulted in a very robust workflow with little or no sample failure. Our present setup, used for prenatal, neonatal and postnatal referrals, is fully automated with respect to sample and data processing (combining both SNV and CNV analysis) and has a turnaround time close to 7 days. To date we have processed more than 800 rapid cWES samples, mostly patient-parent trio's. We will present overall results and discuss choices and challenges with respect to patient inclusion, processing steps, data interpretation, and reporting. The overall yield of the rapid cWES procedure is ~30% irrespective of the patient group (prenatal - neonatal - postnatal). The experience of the past three years clearly shows the excellent clinical utility of a fast cWES track. It also shows this fast track should be offered to anyone in urgent need for a genetic diagnosis.

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P14.092C
Pathway based filtering for rare variant analysis in frontotemporal dementia

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Genome-wide rare variant analysis has become a preferred strategy for disease gene discovery. Several algorithms are available to test association of these variants to disease in a case-control study design. However, achieving the necessary power to detect exome-wide significance in rare variant association studies is challenging due to variant number and sample size limitations. Therefore, additional strategies are needed to prioritize and interpret rare variants in underpowered patient cohorts. Accumulating evidence indicates that the endosomal-lysosomal system is a key mediator in the neurodegenerative process. In search of novel disease genes for unresolved frontotemporal dementia (FTD), we applied a pathway-based approach to prioritize endo-lysosomal pathway related genes with rare variants from in-house generated patient-control exome datasets. Starting from a list of 49 seed genes genetically linked or associated to FTD or related neurodegenerative diseases, we identified first and second layer interaction partners of these known dementia-related proteins. The raw interaction network was then filtered for brain-expressed genes based on GTEx. Following, we selected genes based on endo-lysosomal pathway related Gene Ontology terms and KEGG pathways. This step-wise filtering resulted in 2236 endo-lysosomal pathway related genes to be submitted to gene-burden testing in search of novel candidate genes for FTD. In conclusion, pathway and interactome analysis is a useful approach to mine for genes from high-throughput data.

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P14.093D
Orangutan has all the variants you need: monitoring specificity and sensitivity of diagnostic next-generation-sequencing by establishing orangutan DNA as reference standard

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Evaluating the performance of diagnostic sequencing requires reference samples (RS) with validated genetic variations. In human RS, there will always be numerous exons in any given panel where a sequence variation will not be available for quality control (QC). To overcome this problem we tested orangutan (OrU) as donor for QC reference materials. Since OrU NGS reads have only been obtained from whole genome sequencing (WGS) so far, information of how OrU/human sequence divergence effects targeted enrichment by human capture probes is limited. Here, we applied capture-based whole exome sequencing (WES) panels from different suppliers to the male OrU cell line "EB185-JC" (Sigma-Aldrich). As positive control, the male HapMap sample NA12877 was sequenced in parallel, along with a 1:1 gDNA mixture of EB185-JC and NA12877. The reads were mapped to hg19, to the OrU reference sequence and to a combined hg19/OrU "mix" reference. Using this approach, we could show that OrU performs just as good as the competing human DNA. When mapped to the human reference, the OrU exome provides 903.763 variants (average 4.4 variants/WES target), and many of these would be disease causing if present in a human being. Therefore, OrU DNA can be used to measure both analytical and diagnostic sensitivity for almost every human exon and exceeds the variant variety of NA12877 DNA (average 0.3 variants/WES target) by a factor of 13.5 fold. Therefore, OrU DNA is especially valuable for small, disease-focused NGS panels where human RS fail to provide enough variants for a reliable QC.

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P14.094A
Detection of disease causing repeats by multi-locus CRISPR-Cas enrichment and nanopore sequencing

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With the maturation of long-read sequencing technologies, the applications of genome diagnostic tests in patient care are expanding. A promising application is the sequencing of clinically relevant repeat structures in disorders such as ALS, Huntington's disease, fragile X syndrome, and spinocerebellar ataxias. Increasing repeat units within such a repeat locus are indicative of disease severity and age of onset, and are therefore of clinical importance. Due to the repetitive nature and length (up to several kilobases) it is hard to determine the exact structure of repeats with traditional sequencing technologies. Long-read nanopore sequencing has the potential to overcome this problem by sequencing the entire disease locus including the full (expanded) repetitive region and associated epigenetic modifications.

We present our work on the development and testing of a cost-effective and comprehensive diagnostic test that targets clinically relevant repeat structures in one experiment. The test is based on multiplexed sequence enrichment of ten repeat loci using CRISPR-Cas technology combined with Oxford Nanopore sequencing. We used this approach to sequence controls and patients and demonstrate that our approach is able to effectively enrich and sequence clinically relevant genomic repeat structures. In comparison to our current diagnostic assays we show that the number of repeat units within these loci can be detected with high accuracy.

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P14.095B
Mosaicism in patients with sporadic bilateral retinoblastoma detected by NGS using a RB1 gene panel

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Introduction: Retinoblastoma is an embryonic tumor of the retina diagnosed in children under 4 years of age. Conventional techniques (Sequencing of Sanger and MLPA) allow the detection of 40% of the cases that are carriers of inactivating mutations in heterozygosis in the RB1 gene. It is described that between 10-15% are mosaics for the disease. These techniques are not sensitive enough to

identify variants in low proportion present in these cases. Our objective was to study patients with sporadic bilateral retinoblastoma using massive sequencing techniques (RB1 panel).

Materials and Methods: We designed a RB1 panel (Roche-NimbleGen) with 148,330 bp target sequence covered (83.2% of the RB1 gene) and performed genetic analysis of 10 patients with sporadic bilateral retinoblastoma in whom the mutation has not been identified by conventional techniques.

Results: Genetic analysis with the RB1 panel allowed us to detect mosaicism in the RB1 gene of 3 patients in different percentages: 16.28% (RB1:NM_000321:c.1363C>T:p.R455*), 7.09% (RB1:NM_000321:c.1390+2T>C) and 14.07% (RB1:NM_000321:c.2092_2093del:p.Arg698Alafs*22). 30% of the retinoblastomas analyzed were mosaics for the disease.

Conclusions: By conventional techniques, mutations were identified in 85.6% of patients with sporadic bilateral retinoblastoma. RB1 panel allowed reaching 90% of patients diagnosed and the identification of mosaicism of some patients. The existence of mosaicism can modify the risk of transmission to offspring, as well as the appearance of other tumors in adulthood, its identification is a significant improvement in genetic counseling to families.

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P14.096C
A RNA workflow designed for routine clinical diagnostics

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Introduction: Next generation sequencing (NGS) is commonplace in today's clinical practice. Whole exome sequencing (WES) and whole genome sequencing (WGS) is routinely used for the detection of a wide range of variant types, including single nucleotide variants and copy number variations. Despite advances in the field, most diagnostic NGS workflows suffer from a relatively low diagnostic yield (30-50%). This is partly due to the difficulty of predicting the pathogenicity of genetic variation. RNA sequencing offers a wide range of analyses complementary to WGS/WES: including differential expression analysis, and detection of alternatively spliced transcripts. These analyses may be used to evaluate non-coding variants, as well as to pinpoint affected genes and pathways. Herein we describe an RNA sequencing workflow designed to be used in a routine clinical setting. Furthermore, we demonstrate its usefulness on a wide range of patient cases.

Materials and Methods: The pipeline uses publicly available software and databases to detect and present fusion genes, monoallelic expression, alternative splicing and differential expression. RNA was extracted from blood samples, and neuronal stem cells from patients visiting Clinical Genetics, and the Centre for Inherited Metabolic Diseases at Karolinska University hospital. Paired-end sequences were generated using Illumina technology.

Results: We present our pipeline, as well as our analysis of a variety of patient cases, including down syndrome patients and translocation carriers (germline and somatic).

Conclusions: RNA sequencing is a valuable tool in clinical settings, herein we demonstrate a workflow which we aim to implement in routine diagnostics during 2019.

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P14.097D
RNA sequencing solved the most common but unrecognized pathogenic variant in Japanese nemalin myopathy

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The diagnostic rate for Mendelian diseases by whole exome sequencing (WES)is typically 20%-40%. The low rate is partly because ES misses deep intronic or synonymous variants leading to aberrant splicing. In this study, we aimed to apply RNA sequencing (RNA-seq) to efficiently detect the aberrant splicings and their related variants. Aberrant splicing in biopsied muscles from six nemaline myopathy (NM) cases unresolved by WES were analyzed with RNA-seq. Variants related to detected aberrant splicings were analyzed with Sanger sequencing. Detected variants were screened in NM patients unresolved by WES. We identified a novel deep-intronic *NEB*variant in one case, and another novel synonymous *NEB*variant in three cases. The former variant was observed to be the most frequent among all *NEB*pathogenic variants in normal Japanese populations with a frequency of 1 in 178 (20 alleles in 3,552 individuals), but was previously unrecognized. Expanded screening of the variant identified it in further four previously unsolved nemaline myopathy cases. These results indicated that RNA-seq may be able to solve a large proportion of previously undiagnosed neurological/mucle diseases. Acknowledgements: We appreciate Drs. Eriko

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P14.098A

Evaluation of the QuantSeq method for transcriptome analysis using a cohort of genome clinic patients

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Objectives: Several studies have shown that analysis of the transcriptome by RNA sequencing (RNA-seq) can improve our ability to interpret the functional and clinical importance of the genetic variants identified by Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS). Our aim is to develop an RNA-seq method that can be used in a clinical diagnostic laboratory and identify genetic variants affecting RNA isoforms and gene. To this end, we have been comparing a number of RNA-seq platforms to determine key aspects of their performance.

Methods: The QuantSeq 3'mRNA-seq kit by Lexogen was used to develop an RNA-seq method. The UTR-seq was applied to 134 pediatric clinical blood samples collected through the Genome Clinic (Centre for Genomic Medicine). The samples were collected as part of a cohort of patients who had previously been tested by gene specific panels and microarray and were subsequently analyzed by WGS.

Results: Using our UTR-seq platform, we successfully generated high quality libraries from RNA samples. We determined that 33 of the genes with causative variants were expressed in blood and in 3 of those genes RNA levels may differ significantly in the patient compared to RNA levels from all other patients.

Conclusions: We have developed an automated, scalable and high-throughput RNA-seq platform that can generate robust and reproducible result. Having established our first automated clinical RNA-seq pipeline suitable for gene expression analysis we are now setting up full length RNA-seq that will allow us to capture structural changes in RNA (splice junctions and gene fusions).

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P14.099B

PCR based target enrichment for variant confirmation, gene panels and multiplex PCR sample tracking in a whole exome sequencing workflow

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Targeted PCR-based resequencing is an important application in clinical diagnostics. Using primerXL, we have designed one million PCR assays for high-quality and fragmented DNA, covering the entire human exome. Over 6200 assays for hundreds of clinically relevant genes were wet-lab validated. In addition, over 5000 patient-specific variants were confirmed using pxlence assays. All singleplex PCR assays work under universal conditions and result in equimolar sequencing coverage. Here, we present the compatibility of pxlence assays with multiplex PCR applications. As a first product, we designed and validated a cost-effective and flexible sample tracking test. This primer pool enables fast identification of sample swapping or contamination which may occur in laborious library preparation workflows.

Thirty SNPs were selected based on their minor allele frequency, exonic location and overlap with the capture region of exome enrichment kits. We evaluated three different PCR mastermixes and two library preparation methods, followed by 150 bp paired-end sequencing (MiSeq, Illumina).

The SsoAdvanced PreAmp Supermix (Bio-Rad) resulted in superior homogenous coverage following multiplex PCR. No significant difference in coverage uniformity was observed between the Nextera DNA Flex and the NexteraXT DNA library prep method (Illumina). In virtually all tested DNA samples (n=393), 86.29% of the SNPs had a uniform coverage within 2-fold of the mean. Based on the SNP genotypes, DNA samples could unambiguously be discriminated.

In conclusion, we designed and validated a novel sample tracking test for whole exome or genome sequencing. In principle, this strategy could also be used to design gene panel-specific sample tracking solutions.

C. Frauke: E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; pxlence. **T. Van Laethem:** None. **M. De Smet:** None. **P. Coucke:** None. **E. De Baere:** None. **K. Claes:** None. **B. Menten:** None. **J. Vandesompele:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; pxlence. **L. Steve:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; pxlence.

P14.100C
A new tool CovReport generates easy-to-understand sequencing coverage summary for diagnostic reports

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Gene panel sequencing has become the standard first-tier approach to identify the genetic cause of many diseases. With increased use of these tests, there is a critical need to make the resulting diagnostic reports extremely clear to avoid any misinterpretation. Target sequence coverage is a key quality control information for any diagnostic sequencing test, since important clinical decisions are made based on this data. If coverage is not sufficient even for a small region of a highly suspected candidate gene, a complementary sequencing test is needed to make sure that the pathogenic variant is not missed.

To facilitate the coverage data interpretation by test prescribers, we designed a novel easy-to-use visualization tool, CovReport. The concise coverage data summary generated by CovReport allows one-glance assessment of the sequencing test performance. Moreover, exon-level coverage information can be immediately appreciated and taken into consideration for further medical decisions. CovReport can be easily adapted by any diagnostic laboratory, since it does not require complex installation. The user friendly interface generates a graphic summary of coverage data that is directly amendable to the diagnostic report. In addition to the stand-alone version, we also provide the command line version of CovReport that can be integrated in any bioinformatics pipeline. CovReport is now part of the routine analysis pipeline for all diagnostic tests by gene panels in our Department.

In conclusion, CovReport is a new easy-to-use tool to generate graphic and understandable summary of sequencing coverage data that can be directly amended to the diagnostic report.

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P14.101
Didentifying *SMN1* intragenicmutations usinga newly-developed allelic-specific rt-PCR(as-rt-PCR)method

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Introduction: Spinal muscular atrophy (SMA) is caused by homozygous deletions of the *SMN1* gene in approximately 95% of patients. The remaining 5% of cases have compound heterozygous mutations with one deleted/converted-*SMN1* allele and one mutated allele. *SMN1* mutation detection is complicated by the highly homologous *SMN2* gene, which remains a challenge in clinical practice for diagnosing SMA patients with a mutated allele.

Methodologies: Nine unrelated families that received a SMA molecular diagnosis during 2011-2016 were enrolled in this study. Heterozygous deletions of *SMN1* were detected by quantitative PCR, and suspected *SMN* mutations were scanned through DNA sequencing. A newly developed approach named as allelic specific RT-PCR method (AS-RT-PCR) was used to confirm whether the identified mutation occurred in *SMN1* or *SMN2*.

Results: Six *SMN1* mutations (c.683T>A, c.22_23insA, c.815A>G, c.19delG, c.551_552insA and c.401_402delAG) were identified in 8 families. The latter three are novel mutations that have not been previously reported. Three families carried the same mutation, c.22_23insA. A rare variant c.84C>T in one family found in *SMN2*.

Conclusions: This study demonstrated AS-RT-PCR to be a relatively simple and reliable method for the identification of *SMN1* subtle mutations. Mutation analysis revealed a distinct ethnic specificity in the *SMN1* mutational spectrum and enriched the *SMN1* mutation database.

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P14.102A
Individual assessment for Single Nucleotide Variant

Classification using the Genomics Training, Assessment and Competency Tool (G-TACT)

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The aim of this external quality assessment (EQA) was to provide an online competency assessment for individuals routinely performing interpretation and classification of variants. Participants were required to apply their usual processes to classify five single nucleotide variants (SNVs) and to submit evidence supporting the assigned classification. The variants ranged from class 3, uncertain significance to class 5, pathogenic and included variants in the following genes: *COL4A4, ETFDH, GK, SHOC2* and *SPG7*. The submissions were scored, reported to each participant and a summary report was issued. The report detailed the expected variant classifications and provided an overview of all submitted results. A follow up webinar summarised the evidence assessed, the expected variant classifications and addressed participant queries. This trial scenario was used by 142 individuals, with 82 participants completing the classification of all variants. As G-TACT is web-based then participation is global and 34 countries were represented worldwide. None of the participants provided the expected classification for all five variants, with only 10% of participants providing the expected classification for four variants. This clearly demonstrates the challenges associated with variant classification and the need for education, competency assessment and standardisation worldwide.

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P14.103B

Diagnostic implementation of RNA sequencing in patients lacking a definite molecular diagnosis

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Whole exome sequencing (WES) identifies a causative variant leading to a molecular diagnosis, in only 30-50 % of cases. WES is not effective for the detection of deep, intronic variants that affect pre-mRNA splicing. Furthermore, WES does not provide direct insight into the effects of detected variants on pre-mRNA splicing. Recently, massively parallel mRNA sequencing (RNA-seq) was successfully used to identify pathogenic variants in patients lacking a molecular diagnosis (Kremer et al., 2017; Cummings et al., 2017) and therefore we have implemented RNA-seq in our clinical diagnostic laboratory to help (i) identify pathogenic variants in WES mutation-negative patients and (ii) investigate the effects of variants of uncertain clinical significance (VUS) on pre-mRNA splicing. We will report our results on an initial series of 9 cases. RNA isolated from cultured skin fibroblasts and/or blood was subjected to RNA-seq. In 3 cases we confirmed that the identified VUS affected pre-mRNA splicing, resulting in a more accurate molecular diagnosis. In addition, in 2 cases we identified splicing abnormalities that had not been detected using other technologies. Both cases involved insertions of mobile elements deep in intronic sequences, causing complex mapping issues. Overall, our data suggest that RNA-seq is not only well suited to the detection of intronic variants and rearrangements that are not covered by WES but is also useful for the improved classification of VUS identified using other molecular screens. RNA-seq is a useful adjunct to standard molecular screens, leading to improved diagnostic yields and more accurate variant classification.

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P14.104C

Length and temperature dependent cleavage of TaqMan-type probes

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Due to its high specificity and sensitivity, PCR remains the cornerstone of clinical diagnostics. In particular, applications utilizing a probe-based readout, offer a higher level of specificity given the additional, specific hybridization required to generate a signal.

TaqMan probes are particularly useful for such applications and since their invention over 20 years ago, still comprise the most used probe-type.

TaqMan probes comprise dual-labeled probes, typically labeled with a 5' fluorophore and a 3' located quencher. If the target sequence is amplified during PCR, the TaqMan probe will bind and the 5'->3' exonuclease activity of the progressing Taq polymerase will gradually degrade the probe, releasing the flurophore from the quencher and generating a signal.

Extensive data and design-rules exist for the design of TaqMan probes, however, little emphasis has been put on predicting the specific process of degradation of TaqMan probes. But with the advent of novel designs of TaqMan probes, multiplexing formats and use of e.g. internal quencher types, the length and temperature dependence of the TaqMan degradation process have become increasingly important.

To investigate the dynamics of degradation, we have designed and tested a large number of TaqMan and TaqMan-like designs and show clear dependence on the probe sequence and length as well as assay conditions on the processing of the probes. By using a combination of traditional designs and various internal labeling types we present a set of comprehensive and useful design-rules when the degradation parameters of a TaqMan probe is required to occur within a specific frame.

W.M.M. Echwald: A. Employment (full or part-time); Modest; Anapa Biotech A/S. **S.M. Echwald:** A. Employment (full or part-time); Significant; Anapa Biotech A/S.

P14.105D
Assessing variations in telomere length quantification for epidemiological studies

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Relative telomere length (RTL) has been used as a biomarker for various disease conditions and studies involving telomere biology, unhealthy lifestyle and behavioural choices, stress reduction and mindfulness relaxation programmes, and genetic epidemiology. However, intra- and inter-laboratories inconsistencies in RTL measurements limit the comparison of these values between studies. This study compared the influence of three commonly used DNA extraction kits from Qiagen, namely Blood and Cell Culture Mini Kit (GT), Gentra PureGene Blood Kit (PG) and QiaAmp DNA Blood Mini Kit (QA) on RTL measurements using qPCR. DNA extracted by GT, PG and QA methods show mean RTLs of 1.09 (range 0.67-2.15), 0.83 (range 0.20-1.50) and 0.97 (range 0.64-1.47) respectively. The results indicate that the type of extraction methods can influence mean RTLs (n=65, Wicoxon Signed Rank tests, $p<0.05$) and range of RTLs detected. RTL variance ranged between 0.97 and 0.039 among the three methods. These differences could be attributed to the different underlying principles in DNA extraction for each method. Nonetheless, there was high inter-assay reproducibility in RTL measurements within samples extracted by same method (n=18, PCC=0.846, $p<0.000$) and two-tailed paired T-test showed no significant difference from multiple RTL assays (n=18, T-test pval= 0.65). This study shows that while differences can exist between the telomere lengths measured from different methods, it is important to use the same method of DNA extraction for comparative studies. Reliable and accurate RTL assessment is crucial for epidemiological studies using this biomarker to avoid false positive or negative associations.

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P14.106A
Detection of low fraction mosaics in brain surgical specimens of Type II Focal Cortical Dysplasia with Molecular Inversion Probes

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Introduction: Focal Cortical Dysplasia Type II (FCDII, MIM: 607341) is a localized malformation of cortical development leading to drug-resistant epilepsy that can be treated with surgical resection of the dysplastic area. Hyperactivated MTOR signaling is visible in FCDII, associated in up to 40% of cases with low allele fraction (AF) mosaic gain of function mutations in MTOR itself or other genes in the cascade. Here we describe the implementation of single molecule Molecular Inversion Probes (smMIPs) as an accurate and cost-effective procedure to detect somatic, brain-specific pathogenic variants with AF as low as 1%.

Materials and Methods: DNA was extracted from Formalin Fixed, Paraffin Embedded (FPPE) or Fresh Frozen (FF) samples of forty-five surgically resected FCDII tissues. Whenever possible, matched DNA from non-dysplasia FFPE specimens (gliosis) or from the marginal area of FF tissue were also obtained. We used smMIPS to capture MTOR mutational hotspot (FFPE) or all (FF) exonic sequences and sent samples to Illumina sequencing and bioinformatics analysis.

Results: smMIPs showed median per-base error rate 2.29e-03 (FFPE) and 1.16E-03 (FF). Thirty single or matched samples achieved adequate sequencing coverage

threshold for low AF variant calling. In 23% of samples, we identified and validated hotspot mutations including recurrent p.Ser2215Phe (or p.Ser2215Tyr) and p.Leu1460Pro with allele fraction ranging 3-6%.

Conclusion: smMIPs enable accurate detection of mosaic mutations, yielding diagnostic rate in line with literature. We also highlight technical issues hampering adequate sequence coverage in FFPE samples.

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P14.108C
Variant re-interpretation in the context of familial cascade testing

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Rapid technological advances have led to a substantial increase in the number of sequence variants detected in probands. A direct consequence of this is increased requests from providers to test family members. In familial cascade testing, the targeted testing of a known sequence variant is offered to relatives of the proband to determine their own risks of developing the pathology, and results may impact clinical management. Consequently, a consistent approach to maintain up-to-date variant classification in the context of familial testing is fundamental to provide appropriate patient care and management.

We surveyed 21 clinical laboratories across Canada and the United States to identify variant re-interpretation workflows when performing familial testing. We also analyzed internal data of all familial testing performed by our laboratory in one year.

While most laboratories agreed that ideally all variants should be re-interpreted every time they are detected in a patient, that is not a feasible goal given limited resources. Our survey of laboratories found no clear consensus on when variant re-interpretation is performed; some variables included the variant classification, whether the variant was detected in the family member, and whether the original proband had been tested by the performing laboratory. The review of our internal data showed that of the familial variants re-interpreted (n=107), 11% had a clinically significant change in classification, highlighting the importance of continuing to re-interpret variants tested in this context. We have developed protocols to selectively re-interpret the variants that are most likely to impact patient care.

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P14.109D
A third generation long-read sequencing approach for the analysis of genomic duplication variants, at nucleotide resolution, using Cas9 target enrichment

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With the widespread use of low-cost genome-wide diagnostic screening tests, unanticipated but possibly pathogenic dosage changes affecting single genes are discovered with increasing frequency. Clinical management demands facile validation of such incidental findings, often necessitating the design of custom variant-specific assays. Although deletion variants can be readily confirmed using a range of next-generation sequencing strategies, characterising duplication variants, at nucleotide resolution, remains demanding. We have addressed this challenge by deploying a novel Cas9 enrichment strategy combined with long-read Oxford Nanopore MinIon sequencing. We used bulk genomic DNA without the need for PCR amplification. We present the diagnostic resolution of two problematic cases in which incompletely characterised duplication variants had been identified by array CGH. The first patient presented with learning difficulties and autism spectrum disorder, but had been found to have an incidental 1.7-kb imbalance which included a partial duplication of *VHL* exon 3. This was inherited from the patient's father, who had renal cancer aged 38 years. In the second case, we identified an incidental 200-kb duplication which included *DMD* exons 30-44. Parental testing was consistent with this variant having arisen *de novo*. In both cases, the single-molecule sequencing yielded sufficient information to define precisely the architecture of the rearranged region, enabling Sanger sequencing assays across the integration sites and surrounding homologous regions (that likely gave rise to the duplicated sequences). Adoption of this approach by diagnostic laboratories promises to enable rapid and cost-effective characterisation of challenging duplication-containing alleles.

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P14.110A

Breakpoint detection of balanced and unbalanced structural variants from low coverage WGS data

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Introduction: Structural variants (SVs) are known to be associated with severe genetic disorders. Currently used methods to identify SVs include conventional karyotyping, FISH and microarrays. Yet no method provides both high resolution and identification of balanced variants. We studied the potential of low coverage WGS for fine-mapping breakpoints of SVs by comparing different SV analysis software.

Materials and Methods: Low coverage WGS was performed on 8 samples with previously detected different SVs using KAPA HyperPlus PCR-free library preparation kit (Roche) and NextSeq 550 (Illumina) sequencing platform. Mean genome coverage was 8.5X. Breakpoint analysis was done by manual genome exploration using IGV software and by automatic SV callers: VarSeq (Golden Helix) and Delly2.

Results: Delly2 software predicted breakpoints for all 8 known SVs including complex variants. VarSeq aided in finding better defined breakpoints than microarray for all SVs with copy-number variations, however it was not suitable for balanced events. Furthermore, by manually exploring the region of interest using IGV we could provide better characterization of known SVs and pinpoint breakpoints at single-base precision.

Conclusion: Using currently available software, it is possible to detect breakpoints at single-base level if the approximate SV region is known. However, the best results were achieved when called breakpoints were manually inspected on genome visualization software. For now, SV analysis from low coverage WGS data could be used to supplement conventional methods where identifying a precise breakpoint can provide clinically relevant information.

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P14.111B

Whole exome sequencing and parallel biochemical analyses: an efficient combination for the diagnosis of genetic diseases

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Introduction: Whole exome sequencing is an efficient tool for the study of monogenic disorders. However, inconclusive results are often obtained due to the lack of specific clinical information and/or the identification of variants of uncertain significance, which do not allow to confirm a genetic diagnosis in the absence of further testing. As a result, the "diagnostic odyssey" for patients may be significantly extended in time. This study focuses on the validation of genetic results by means of biochemical analyses.

Materials and Methods: Whole exome sequencing was performed for 24 patients coming from different geographical regions. The results obtained by sequencing were validated in parallel through biochemical analyses: quantification of the corresponding enzyme activity and/or biomarker.

Results: Sequencing analyses revealed the presence of variants with known clinical relevance (previously reported in the literature) and previously undescribed variants in 14 genes associated with lysosomal storage disorders. The combination of genetic and biochemical results confirmed the pathogenicity of previously undescribed variants and the pathogenicity of those variants previously known. In two cases, the diagnosis was confirmed despite the lack of specific clinical information and with only the detection of intronic variants, which would have otherwise been classified as variants of uncertain significance.

Conclusions: The whole exome sequencing approach can be greatly reinforced by performing biochemical analyses in parallel. The combination of these two methods increases the diagnostic yield of genetic disorders even at initial stages in which the clinical signs are still unspecific. This may also allow starting early pharmacological/non-pharmacological treatments.

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P14.112C

Whole genome sequencing reveals complex chromosome rearrangement disrupting *NIPBL* in infant with Cornelia de Lange syndrome

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Introduction: Routine diagnostic work-up of children with suspected monogenic disorders often includes chromosomal microarray and next-generation sequencing, and foregoes classical cytogenetic analysis that can detect balanced translocations. We identified an infant with Cornelia de Lange syndrome (CdLS) who manifested with typical dysmorphic facies and limb reduction defects, yet chromosomal microarray and exome sequencing from both whole blood and buccal samples were noncontributory.

Materials and Methods: G-banding chromosome analysis was followed by PCR-free whole genome sequencing (WGS) at an average read depth of 30X. WGS data was utilized to fine map the rearrangement breakpoints. The disease-associated breakpoint junction was validated by breakpoint junction PCR.

Results: Chromosome analysis revealed a *de novo* complex balanced translocation, 46,XY,t(5;7;6)(q11.2;q32;q13)dn. The reported cytogenetic breakpoints did not involve any of the known CdLS-associated genes, prompting us to perform breakpoint analysis by WGS. Twenty six breakpoints were identified by WGS, delineating segments derived from four chromosomes (5;6;7;21) in ancestral or inverted orientation. One of the breakpoints disrupted *NIPBL*, on 5p13.2, providing molecular correlation for the clinical diagnosis of CdLS.

Conclusion: WGS provides a unique opportunity to unravel the basis of genetic disorders not resolved by routine laboratory methods, and to shed light on the genomic mechanisms involved in the pathogenesis. The rearrangements identified in this case were by far more complex than what was suggested by traditional G-banding chromosome analysis, allowing for identification of the underlying molecular diagnosis and implicating chromothripsis and chromoanasynthesis in the formation of the balanced translocations.

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P14.113D

From cytogenetics to cytogenomics: whole genome sequencing as a comprehensive genetic test in rare disease diagnostics

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Rare genetic diseases are caused by different types of genetic variants, from single nucleotide variants (SNVs) to large chromosomal rearrangements. Recent data indicates that whole genome sequencing (WGS) may be used as a comprehensive test to identify multiple types of pathologic genetic aberrations in a single analysis.

We present FindSV, a bioinformatic pipeline for detection of balanced (inversions and translocations) and unbalanced (deletions and duplications) structural variants (SVs). First,

FindSV was tested on 106 validated deletions and duplications with a median size of 850 kb (min: 511 bp, max: 155 Mb). All variants were detected. Second, we demonstrated the clinical utility in 138 monogenic WGS panels. SV analysis yielded 11 diagnostic findings (8%). Remarkably, a complex structural rearrangement involving two clustered deletions disrupting *SCN1A*, *SCN2A*, and *SCN3A* was identified in a three months old girl with epileptic encephalopathy. Finally, 100 consecutive samples referred for clinical microarray were also analyzed by WGS. The WGS data was screened for large (>2 kbp) SVs genome wide, processed for visualization in our clinical routine arrayCGH workflow with the newly developed tool vcf2cytosure, and for exonic SVs and SNVs in a panel of 700 genes linked to intellectual disability. We also applied short tandem repeat (STR) expansion detection and discovered one pathologic expansion in *ATXN7*. The diagnostic rate (29%) was doubled compared to clinical microarray (12%).

In conclusion, using WGS we have detected a wide range of structural variation with high accuracy, confirming it a powerful comprehensive genetic test in a clinical diagnostic laboratory setting.

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P14.114A
Detection of copy number variants using whole-exome sequencing improves diagnostic yield of patients with rare Mendelian diseases

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Copy number variants (CNVs) are commonly observed in human populations and increasingly recognised as an important aetiology of disease. Whole-exome sequencing (WES) has become a robust and cost-effective approach for clinical genetic testing of small sequence variants; detection of CNVs within WES data became possible with the development of algorithms that enable comparing coverage between probands and controls. In this study, we express the challenges and feasibility of analysing CNVs using WES data. We performed WES on patients with rare genetic disorders and analysed SNVs, using an in-house pipeline, and CNVs, using Golden Helix's VarSeq software. Probable causative CNVs were confirmed by qPCR or MLPA. In recessive disorders with a causative heterozygous SNV, fine-tuning of CNV analysis for each gene was performed on a case-by-case basis. During 2017, we detected 12 CNVs as probably causative in 122 patients, but only one was confirmed by qPCR, a rate of 91.7% false positives (FPs). After optimising regions of interest and using CNVs of healthy controls, from public and internal CNV databases, many common CNVs and FPs were filtered. Then, over the past year, we detected 123 CNVs in 1251 patients as likely causative, 41 of which were confirmed by qPCR or MLPA (66.7% FPs). We identified probable disease-causing CNVs using WES data in 3.3% of patients. Confirmation by orthogonal methodologies validated the software analysis pipeline and the CNVs detected, showing that combining SNV and CNV analysis improves the molecular diagnosis of patients with rare Mendelian diseases.

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P14.116C
Increased efficiency with a new SNP panel for zygosity testing

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A requirement for performing robust genetic and statistical analyses on twins is correctly assigned zygosities. We have developed a new and improved SNP panel for zygosity analysis, genotyping 37 autosomal SNPs and 2 sex markers on the zinc finger X/Y chromosomes, using the Agena MassARRAY® system. The 37 SNPs were previously included in a larger panel, to date used for zygosity analysis in 12800 twin pairs from the Swedish Twin Registry. The goal for the new panel was to develop a one-reaction assay, to replace the larger three-reaction assay, containing targets with the highest minor allele frequencies based on results from 25000 investigated samples.

Saliva DNA from 310 twin pairs was analyzed using both the new panel and the larger original panel. The genotypes were 100% concordant between the two panels. The sex markers scored 100% correctly in all runs. The zygosity score was calculated using odds ratios of monozygosity (MZ), taking into account an error rate of 0.1%. Odds ratios for MZ score using the new panel and the original panel was greater than $6x10^6$ and $6x10^7$, respectively. Odds ratios for dizygotic score (DZ) was 0 for both panels. Using the new panel, 308 of the 310 twin pairs could be analyzed while 304 twin pairs could be analyzed with the original panel.

We found that a new panel could replace a well-used panel for zygosity analysis. The new panel is faster and easier to run, since it only uses one well per sample.

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P15
Personalized/predictive medicine - Pharmacogenomics

P15.01D
CRISPR-Cas9 in Alport Syndrome: HDR in podocytes-lineage cells as new therapeutic frontiers in an untreatable disorder

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Alport syndrome (ATS) is a clinically heterogeneous nephropathy characterized by Glomerular Basal Membrane abnormalities up to end-stage renal disease. ATS therapies are currently only delaying the progression of symptoms.

We present here a stable approach using AAV-CRISPR/Cas9 technology. We have recently proven that podocytes-lineage cells - the key cells in ATS pathogenesis, the only ones to express COL4 α3-α4-α5 heterotrimer - can be isolated from urine samples of ATS patients. Taking advantage of disease-relevant cell lines, we employed an two-plasmid approach in order to achieve a mutation-specific correction, suitable for AAV infection. The first plasmid carries a Donor DNA, the template for the correction, and a reporter system mCherry/GFP to track the activity of Cas9 in the cells. The second plasmid carries a self-cleaving SpCas9 and the mutation-specific sgRNA. In two stable podocytes-lineage cell lines, harboring a mutation in *COL4A5* (p.(Gly624Asp)) and a mutation in *COL4A3* (p.(Gly856Glu)), we achieved a reversion of the mutation greater than 30% and insertions/deletions, limited to the mutated allele, lower than 40%. AAV2 turned to be the most effective serotype for *in-vitro* infection using a viral MOI of 10^5. *In-vivo* gene editing experiments, are ongoing on a naturally occurring dog model. The described approach, covered by patent 102018000020230, opens up a new era in the treatment of ATS, through an AAV personalized corrective approach specifically designed for each patient.

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P15.04C
A genome-wide functional genomics approach identifies susceptibility pathways to fungal bloodstream infection in humans

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Introduction: Candidaemia, one of the most common causes of fungal bloodstream infection, leads to mortality

rates up to 40% in affected patients. Understanding genetic mechanisms for candidaemia susceptibility may aid in designing host-directed therapies.

Materials and Methods: We performed the first genome-wide association study on candidaemia using the largest patient cohort available today. By using a population-based cohort of 500 healthy volunteers, we profiled cytokines in *Candida*-stimulated peripheral blood mononuclear cells, whole blood, and macrophages. By using these cytokine profiles and genetic data, we mapped *Candida*-response cytokine-QTLs, and tested if they are associated with susceptibility. Finally, we tested if candidaemia loci modulating cytokines have an impact on the production of reactive oxygen species (ROS) by using an independent population-based cohort.

Results: We observed strong association between candidaemia and a genetic variant, which significantly affects the expression levels of *PLA2G4B* in blood. We found that up to 35% of the susceptibility loci affect in vitro cytokine production in response to *Candida*. Furthermore, potential causal genes located within these loci are enriched for lipid and arachidonic acid metabolism. We also showed that the numbers of risk alleles at these loci are negatively correlated with ROS and IL-6 levels in response to *Candida*. Finally, there was a significant correlation between ROS and allelic scores based on 16 independent candidaemia-associated SNPs that affect monocyte-derived cytokines, but not with T-cell derived cytokines.

Conclusions: Our results prioritize the disturbed lipid homeostasis and oxidative stress as potential mechanisms that affect monocyte-derived cytokines to influence candidaemia susceptibility.

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P15.05D

Genetic variants predisposing to common diseases in whole exomes of Bulgarian healthy individuals

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Introduction: Common diseases are a serious cause of mortality worldwide and in Bulgaria also. Centenarian's genomes/exomes contain clues to clarify the role of the genes with unambiguous clinical significance. The aim of our study is to investigate and compare the frequencies of variants in BG centenarians/controls predisposing to common diseases used in different panel tests in medical genetic counseling.

Materials and Methods: Two pools were constructed: one with DNAs from 31 centenarians and the other with DNAs from 61 young and healthy Bulgarian individuals. WES analysis was performed and 177 130 variants were found. We selected 79 variants considered as predisposing to common diseases: cardiovascular, inflammation, diabetes, etc, from the commercially available genetic tests.

Results: From the 79 genetic variants 33 were identified in our samples. Seven variants are associated with susceptibility to common diseases (ClinVar and Marrvel): obesity (*UCP2* rs660339- 0,314 in centenarians and 0,460 in controls); apolipoprotein C-III deficiency (*APOC3* rs5128 0,925 and 0,847); MTHFR thermolabile polymorphism (*MTHFR* rs1801131; 0,280 and 0,396); hypertension, essential (*AGT* rs699; 0,351 and 0,426); metabolic syndrome, asthma, nocturnal (*ADRB2* rs1042713; 0,380 and 0,329); coronary artery spasm 1 (*NOS3* rs1799983; 0,713 and 0,676); neural tube defects, folate-sensitive (*MTR* rs1805087; 0,195 and 0,175).

Discussion: The determination of predisposition to common diseases requires analysis of the genetic profile in the context of the environmental impact. Further more comprehensive research is needed to clarify the clinical significance of the remaining variants. Acknowledgment to DN 03/7 from 18.12.2016 - National Science Fund of Bulgaria

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P15.06A

Response to adalimumab: can a genetic prediction profile differentiate between responders and non-responders in Slovenian Crohn's disease patients?

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Introduction: Non-response to anti-TNF therapeutics such as infliximab and adalimumab usually represents loss of disease control in severe Crohn's disease (CD). We aimed to investigated whether a previously reported gene profile for infliximab could also be applied to adalimumab response.

Materials and Methods: Inflamed and non-inflamed colon biopsy samples from 47 Slovenian Crohn's disease patients indicated for adalimumab (ADA) were obtained during routine colonoscopy prior to ADA treatment. Response to adalimumab was determined using IBDQ. RT-qPCR was employed to measure gene expression in colon biopsy samples. Genotypes were extracted from previously available data. Statistical analysis was performed with SPSS software. Bootstrap aggregated support vector machines (SVM) were trained using R package e1071.

Results: Pooled, non-inflamed colon tissue and inflamed colon tissue gene expression datasets were used to train the SVM prediction models using IBDQ response after 4, 12, 20 and 30 weeks of ADA treatment as prediction target. Pooled and inflamed datasets achieved an average of 75.5% and 90.5% ADA response prediction accuracy, respectively. Non-inflamed tissue dataset achieved 100% ADA response prediction accuracy. Moreover, SVM trained on selected SNPs and LD mates from analyzed genes had an average accuracy of 92.8%, confirming involvement of analyzed genetic regions. Finally, combined expression and genotype data showed 100% ADA response prediction accuracy for all datasets.

Conclusions: Our results support the reported genetic anti-TNF response profile. Furthermore, it can be applied for adalimumab prediction, warranting further targeted genetic profiling for response to adalimumab therapy.

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P15.07B

Missense variant rs3740691 in gene *ARFGAP2* predicts short-term nonresponse to anti-TNF inhibitor adalimumab in Crohn's disease patients

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Introduction: In several autoimmune complex diseases, an increased secretion of proinflammatory cytokine tumour necrosis factor (TNF) plays an essential role in the initiation and propagation of the disease. Therefore, anti-TNF monoclonal antibodies have shown an increased efficacy over conventional therapies. However, therapy with TNF inhibitors is ineffective in up to 30% of patients and the variety of therapeutic effects may reflect individual genetic backgrounds of patients. The aim of our study was to find new pharmacogenetics markers and mechanisms of response to anti-TNF inhibitor adalimumab in well-defined cohort of Crohn's disease (CD) patients.

Materials and Methods: We enrolled 102 CD patients on adalimumab for which response has been defined after 4, 12, 20 and 30 weeks of treatment. SNPs, previously associated with response to anti-TNF treatment, were genotyped. Functional prediction has been performed for significantly associated genes.

Results: After four weeks of treatment, strong statistically significant association has been confirmed for SNP rs3740691 in gene *ARFGAP2*. In a group of patients with genotype AA or AG there were 59,6% of nonresponders compared to 15,1 % of nonresponders in a group of patients with genotype GG (p= 1,20E-05). Furthermore, after four weeks of treatment, average IBDQ value in patients with genotype AA or AG reached only 158,3 points compared to 183,7 points in patients with genotype GG (p=2,74E-04). The difference remained significant also after 12 weeks of treatment.

Conclusions: This is the first report that supports the association of SNP rs3740691 in gene *ARFGAP2* with response to adalimumab in CD patients.

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P15.08C

Plasma circulating tumor DNA as a genomic biomarker for ovarian cancer

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Introduction: Ovarian cancer (OC) remains the most lethal disease among gynecological malignancies as most patients are diagnosed in an advanced stage. Circulating tumor DNA (ctDNA) liquid biopsy analysis holds promise as a minimally-invasive multipurpose biomarker. In this pilot study, we investigate ctDNA monitoring in OC.

Material and Methods: Twelve OC patients from Keio Women's Health Biobank at Keio University School of Medicine were studied: the histological subtypes comprised eight serous, two clear cell, one mucinous and one endometrioid. Diagnostic tumor and serial peripheral blood samples were collected. Following identification of tumor mutations, we performed quantitative detection of ctDNA in plasma from various timepoints using the ultrasensitive IBSAFE method.

Results: Concordant mutations in tumor DNA and plasma DNA were detected in eleven patients (91%); only one mutation in a non-recurrent patient was undetectable in plasma. Among the five recurrent patients, all patients detected positive ctDNA prior to or simultaneously to CT imaging. Positive ctDNA was detected earlier than high CA125 in 3/5 patients with a median of 86 days. ctDNA also had sensitive reaction to tumor burden during chemotherapy. ctDNA from all seven non-recurrent patients turned negative after initial treatment and maintained negative throughout the follow-up.

Conclusions: Detection of plasma ctDNA was feasible. ctDNA has the potential of enhancing presence diagnosis by shortening the lead time than CA125 or CT imaging. ctDNA may be a clinically useful biomarker for OC patients.

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P15.09D
The *CYP2D6* gene variation among three Croatian Roma groups

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Introduction: CYP2D6 enzyme is involved in the metabolism of approximately 25% of the most commonly prescribed drugs metabolized in liver (opioids, beta-blockers, antidepressants, antitumor agent, etc.). *CYP2D6* has the largest phenotypical variability among all CYP's due to the substantial number of polymorphisms that impact its activity. This gene shows population specific variation. Aim: to determine variation within CYP2D6 among three socio-culturally and geographically distinct Croatian Roma groups (Balkan Roma and Vlax Roma from Baranja and Medjimurje) since their specific population history highly influenced their gene pool.

Methods: NGS method Genotyping-in-Thousands by sequencing was used to sequence whole *CYP2D6* gene on 324 Croatian Roma DNA samples. Polymorphic positons were phased using PHASE v2.1.1. Reconstructed haplotypes were translated into star alleles according to P450 Allele Nomenclature Database.

Results: Sequencing identified 51 polymorphic positions. Reconstructed haplotypes significantly differed among the three Roma groups ($p<0.05$). The two most frequent haplotypes in Balkan Roma belong to the groups of CYP2D6*4 and CYP2D6*1 alleles (9.7% and 8.7% respectively), while in Roma from Baranja the most frequent haplotypes belong to groups of CYP2D6*1 and CYP2D6*2 alleles (18.8% and 10.3% respectively). The most frequent haplotypes in Roma from Medjimurje belong to the groups of CYP2D6*1 and CYP2D6*4 alleles (14.4% and 7.4% respectively).

Conclusion: Our results provided basic information about *CYP2D6* polymorphisms, suggesting that the enzymatic activities of *CYP2D6* might differ among Roma groups. This finding may be helpful for better modulation of pharmacotherapy in Roma population. The research was funded by Croatian Science Foundation grant (HRZZ-IP-2014-09-4454) to MPS.

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P15.11B
Exome sequencing allows detection of relevant pharmacogenetics variants in epileptic patients

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Introduction: Beyond the identification of pathogenic variants for the diagnosis of Mendelian disorders, sequencing of individual genomes can detect numerous variants potentially relevant for clinical care. Clinical interventions can thus be conducted to improve future health outcomes in patients and their at-risk relatives, such as predicting late-onset genetic disorders accessible to prevention or treatment, or identifying differential drug efficacy and safety.

Material and Methods: To evaluate the interest of pharmacogenetics information, we designed an « in house » pipeline to determine the status for 122 PharmGKB (Pharmacogenomics Knowledgebase) variants, including structural variations for the genes of interest. This pipeline was applied in a cohort of 82 epileptic patients to determine the frequency of pharmacogenetics variants of interest for managing the treatment. Plasma concentrations and treatment was retrospectively assessed by clinical history.

Results: For the PharmGKB class IA variants, the *CYP2C9* status for phenytoin's prescription was the only relevant information. One low and twenty-three intermediate metabolizers were identified respectively in our cohort, nineteen patients were treated by phenytoin. While being treated with a standard protocol (15mg/kg loading dose followed by 5mg/kg maintenance dose), 3 out of the 4 identified intermediate metabolizers had experienced plasma concentration above the toxic range (30 mg/L).

Conclusion: Genotyping of *CYP2C9* could have anticipated the risk of clinical toxicity caused by high phenytoin plasma levels. Pangenomic sequencing can provide information about common pharmacogenetic variants in epileptic patients likely to be useful for their pharmacological management.

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P15.12C

Identifying undetected Familial Hypercholesterolaemia (FH) in the general population - perspectives of primary health care professionals in the UK

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Introduction: Familial hypercholesterolaemia (FH) is one of the most common inherited autosomal dominant disorders, causing elevated low-density lipoprotein (LDL) cholesterol levels. Left untreated this causes premature coronary heart disease and mortality yet most cases remain undiagnosed. Early detection and effective preventive intervention is a national priority in the UK and other countries. This research formed part of a prospective evaluation of the clinical utility and acceptability of a new FH case finding tool (FAMCAT) applied to patient records in routine clinical primary care practice.

Materials and Methods: Qualitative semi-structured interviews (n=20) were carried out with a purposeful sample of primary care health professionals (GPs, nurses, heath care assistants) and practice managers selected from 10 UK primary care practices who had implemented and used the FAMCAT tool. Following case finding of patients at highest risk of FH, practices either initiated genetic testing on site or referred to other services. All interviews were transcribed and analysed thematically.

Results and Conclusions: Primary care practitioners recognised and welcomed the benefit of adopting a more systematic approach to identification of patients with undetected FH in their practice populations. They found application of the FAMCAT tool was acceptable and feasible in practice, and that genetic testing could be realised on site.

However, implementing more routine identification of, and genetic testing for, FH in primary care should ensure adequate provision is made for increases in workload and resource use whilst taking into account the local organisational infrastructure at each primary care practice.

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P15.13D

Plasma levels of Lyso-Gb1 correlate with HPO term-based estimates of disease severity in Gaucher disease

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Introduction: Gaucher disease (GD) is a lysosomal storage disorder which is highly variable as regards age at onset and clinical manifestation. An enzymatic assay has long been

used as a rather reliable diagnostic aid in GD, but we recently revealed even better diagnostic performance of dried bloods spot (DBS)-based quantification of the sphingosine Lyso-Gb1. We were therefore interested in determining whether this biomarker can also be used to reflect disease severity.

Materials and Methods: A large cohort of individuals with genetically confirmed GD was compiled from Centogene's proprietary mutation database CentoMD®. Free-text clinical descriptions from the initial diagnostic requests (i.e. before genetic diagnosis and before initiation of treatment) were translated into human phenotype ontology (HPO) terms. Lyso-Gb1 levels in the corresponding samples were quantified by mass-spectrometry.

Result: A median of 4 HPO terms (range 1 to 19) had been assigned per patient. Biomarker levels were lowest in patients with a single HPO term, and increased by ~15% per additional term (up to n=5 terms). Stratification of terms according to clinical categories revealed that patients with mild or sub-clinical corresponding signs (e.g. thrombocytopenia) had lower Lyso-Gb1 levels than patients with a more severe manifestation (e.g. thrombocytopenia + excessive bleeding, thrombocytopenia + epistaxis).

Conclusion: In addition to its diagnostic value, Lyso-Gb1 accurately reflects disease severity in GD. Considering feasibility of DBS-based quantification, it thereby lends itself as the ideal biomarker for long-term follow up and therapeutic monitoring of GD patients.

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P15.14A
Genomic Variants and Precision Medicine (personalized clinical Care) in Saudi Arabia

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Saudi Arabia has high rate of consanguineous marriages (around 60%) resulting in inherited disorders early in life affecting ~8% of births. Common diseases including diabetes, cardiovascular disease, and others manifesting later in life affect over 20-30% of the population. In our experience in over 25 years using genomic variant has helped in implementing prevention programs including pre-implantation genetic diagnosis, prenatal diagnosis, newborn screening and pre-marital screening. It has also helped us in establishing personalized therapeutic strategies. The Saudi Human Genome Program targets the sequencing of 100,000 samples. It has sequenced over 25,000 samples from patients and family members with inherited diseases. This has resulted in a large Knowledge Database of genetic variants which identifies pathological and normal variants present in the Saudi population. This database was used to build two proprietary "custom microarrays". The first is the "Saudi Biobank Array" which may be used to study rare inherited diseases and common disorders. The second is a "premarital screening array". These include diseases that result in intellectual disability, inborn errors of metabolism, hearing and vision impairment, immunodeficiency, movement disorders, among others. Based upon our experience within the current pre-marital screening program, approximately 60% of couples with pathological variants, choose not to proceed with marriage. This save healthcare cost of more than SAR 3 billion on an annual basis with reduction in social burden to the community. Therefore establishing the SHGP was a must to provide the necessary infrastructure to solve cases to implement prevention and therapeutic strategies.

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P15.15B
NEBNext Direct Custom Ready Panels overcome challenges associated with targeted re-sequencing

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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 design challenges associated with panel design present challenges. NEBNext Direct Custom Ready Panels employ a novel approach to selectively enrich nucleic acid targets

ranging from a single gene to several hundred genes, without sacrificing specificity. The approach hybridizes both strands of genomic DNA, prior to any amplification. Following a 90-minute hybridization, captured molecules are converted into Illumina sequencer compatible libraries containing an 8 bp sample ID and a 12 bp Unique Molecular Identifier (UMI). An optional dual index module incorporates a second 8 bp sample ID for highly-multiplexed sequencing or to mitigate effects of "pad-hopping" on certain Illumina instruments. The result is a 1-day protocol that enables preparation of sequence-ready libraries from purified genomic DNA specific to content included in the panel. NEBNext Direct Custom Ready Panels can be customized to include the full exon content of human genes associated with cancer, neurological disorders, autism, cardiovascular disease, and other conditions. Baits for these genes have been designed, balanced, and pooled on a per gene basis, and can be combined into specific custom gene subsets. Here, we demonstrate the capability to deploy custom gene panels across panel sizes and content, while maintaining high specificity, uniformity of coverage, and sensitivity to detect nucleic acid variants from clinically relevant samples.

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P15.16C
Cytotoxic and genotoxic evaluation of escitalopram oxalate, a commonly used antidepressant

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Introduction: Escitalopram oxalate (EO), is one of the most prescribed antidepressants in Turkey and in several other European countries, but there is not enough research on the effects of EO at a cellular level, therefore, we aimed to investigate the effects of EO at the cellular and DNA level.

Materials and Methods: The cytotoxic effect of EO was determined by methylthiazolyl diphenyl-tetrazolium bromide (MTT) test in a dose dependent manner. The genotoxic effect of escitalopram oxalate at DNA level was investigated by UV-Vis spectrophotometer and agarose gel electrophoresis employing Copper reduction test.

Results: In MTT assay, EO showed cytotoxic effect on NIH3T3 cells at a concentration of 50 μM. The absorption value of the UV-Vis spectrophotometer solution of EO at 238 nm is increased by the addition of DNA and showed a hyperchromic effect with DNA binding value (Kb) of 0.035 M^{-1}. Moreover, The $CuCl_2$ / $2H_2O$ added to the DNA with EO, and no fracture was observed in the double stranded DNA compared to the control DNA.

Conclusion: In conclusion EO has a dose dependent cytotoxic effect but no genotoxic effect. This study has been supported by Cukurova University Scientific Research Fund. Project Number: FYL-2017-9464 (Adana, Turkey).

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P15.17D
Determining genetic predispositions using CleanPlex®Hereditary Cancer Panel for a rapid and streamlined amplicon-based NGS workflow

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Introduction: Genetic testing being widely accepted; medical professionals are increasingly interested in screening for hereditary conditions. Popularly, hybrid capture-based sequencing methods are used for screening large number of targets, which tend to be tedious, laborious, requiring trained operators with specialized equipment and the complex protocols resulting in increased errors. To simplify screening, we developed amplicon-based CleanPlex® technology, which enables high-quality genomic coverage using a fast, simple, and streamlined NGS library preparation workflow. We present our Hereditary Cancer panel to quickly detect mutations associated with increased hereditary cancer risks, covering full exons of 37 targeted genes in one reaction.

Methods: CleanPlex technology has a 3-step workflow: targeted multiplex PCR step, background cleaning step to remove by-products, and finally indexing PCR to add Illumina® adapter sequences and sample indexes. 10 ng of input genomic DNA per pool (40 ng total) was used and libraries sequenced at ~2,500 read depth using Illumina NextSeq®. After demultiplexing, mapping-rates and on-target rates were calculated, and variants were identified using Paragon Genomics' variant calling algorithm.

Results: The results exhibit >96% uniformity at 0.2X mean, and all exons were covered to provide accurate variant calling. The assay is highly reproducible and sensitive, with an R2 value of 0.95 between independently prepared replicates, and a detection rate of >99% for single nucleotide variants.

Conclusion: CleanPlex Hereditary Cancer Panel can be used for rapid and accurate profiling of cancer risk with very small DNA input. This panel, with an easy cost-effective workflow and quick turnaround, can make genetic monitoring efficient.

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P15.19B
African-specific pharmacogene variants affect rosuvastatin pharmacokinetics in Africans

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Introduction: Rosuvastatin is used in the treatment of hypercholesterolemia. Elevated plasma levels of rosuvastatin have been associated with increased risk of statin induced myopathy, leading to non-adherence and compromising treatment and management of hypercholesterolemia. Pharmacogenetics plays a well-established role in both interindividual and interethnic variation in rosuvastatin plasma levels. Historically the pharmacogenetics of rosuvastatin has been dominated by the transporter genes, *Solute Carrier Organic Anion Transporter Family member 1B1* (*SCO1B1*) *ATP Binding Cassette G2* (*ABCG2*). The single nucleotide polymorphisms *SLCO1B1* c.521T>C (rs4149056, p.Val174Ala) and *ABCG2* c.421C>A (rs2231142, p.Gln141Lys), in particular are associated with increased plasma levels of rosuvastatin. However, these pharmacogenetic studies had minimal representation from individuals of African descent.

Materials and Methods: To investigate the pharmacogenetics of rosuvastatin in African populations; we first screened 785 individuals from nine ethnic African populations for the *SLCO1B1* c.521C and *ABCG2* c.421CA variants. We then sequenced whole exomes from individuals of African Bantu descent, who participated in a 20mg rosuvastatin pharmacokinetic trial in Harare Zimbabwe.

Results: Frequencies of *SLCO1B1* c.521C ranged from 0.0% (San) to 7.0% (Maasai), whilst *ABCG2* c.421A ranged from 0.0% (Shona) to 5.0% (Kikuyu). Novel genetic variants showing significant association with rosuvastatin exposure were identified in *SLCO1B1, ABCC2, SLC10A2, ABCB11, AHR, HNF4A, RXRA* and *FOXA3* and appear to be African-specific.

Conclusions: The variants *SLCO1B1* c.521C and *ABCG2* c.421CA may play a minor role in interindividual variation in rosuvastatin pharmacokinetics in African individuals. Instead, African specific pharmacogenetic variants may play a major role.

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P15.20C
Novel polycistronic modified mRNA for generation of human induced pluripotent stem cells

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Introduction: Cell biology and personalized regenerative medicine are enormously influenced by induced pluripotent stem cells (iPSCs). Modified mRNAs (mmRNA) has shown great potential to derive safe and high efficient integration-free iPSCs. However, low stability of mRNAs resulted in daily transfection, to overcome this limitation, we have constructed a polycistronic mmRNA containing WPRE

element, which improves the half-life of the produced mRNA in the target cells.

Materials and Methods: Four human pluripotency transcription factors along with EGFP were developed as a single expression cassette through using 2A peptide sequence. WPRE was cloned in downstream of described polycistronic fragment, in a separate construct. These two fragments were subcloned in an appropriate vector containing T7 promoter, UTRs and poly-A tail for *in vitro* transcription (IVT). Transcripts were produced by T7 RNA polymerase, using modified nucleotides and cap analogs. mmRNAs were transfected into target cells and EGFP expression was assessed by fluorescent microscopy and flow cytometry. The expression level of reprogramming factors was determined by Western blotting.

Results: Analysis of transfected cells showed significant improvement in mmRNA stability in presence of WPRE, which in turn gave rise to a higher level of protein expression. Western blot assay demonstrated an equivalent stoichiometric expression of 2a-mediated factors.

Conclusions: We developed a stabilized polycistronic mmRNA as a potential tool for safe and efficient iPSCs induction. The application of cap, modified nucleotides and poly-A tail in the structure of mRNA, besides, utilized WPRE element in the expression cassette, enhanced the mmRNA half-life and increased its translation efficiency.

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P15.21D

New genetic variants and pathways associated with healthy longevity in Bulgarian centenarians

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Around 25% of human longevity is attributed to genetic factors. Studies have shown that complex interaction of many variants with small effect and interplay of different pathways predispose to healthy ageing. We performed deep whole-exome sequencing of a pool of 32 centenarians and 61 young healthy controls from Bulgaria and after bioinformatic and statistical analysis we discovered 91 variants showing statistically-significant allele frequency difference between the two pools (p-value<5.0x10-8). Based on their molecular function we nominated three of these variants for association with longevity - rs2526374 in *RNF43* (NP_060233.3:p.Leu418Met), rs956868 in *WNK1* (NP_001171914.1:p.Thr1316Pro) and rs2276362 in *NADSYN1* (NM_018161.4:c.264-26A>G). *RNF43* is a regulator of WNT signalling - an important, highly-conserved pathway involved in cell proliferation, tissue homeostasis and stem cell maintenance in adults. *WNK1* is a serine/threonine kinase that plays an important role in cell signalling, survival and proliferation. This gene is a downstream effector of insulin/IGF-1 signalling - a pathway involved in the regulation of ageing in many organisms. Moreover, *WNK1* modulates the activity of the pro-longevity transcription factor *FOXO4*. Splicing prediction algorithms predict that this intronic variant in *NADSYN1* would disrupt the consensus branchpoint between exons 3 and 4. This gene regulates NAD+ metabolism - an important cofactor involved in lifespan extension in some model organisms and essential for the activity of sirtuins and *FOXO3A* associated with ageing. Our whole-exome pool analysis discovered novel genes involved in longevity that could serve as potential therapeutic targets for the prevention or treatment of age-related physiological decline.

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P15.22A

Personalized approach to the prevention and management of colorectal cancer illustrated on family case of Lynch syndrome

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Introduction: Lynch Syndrome (LS) is a hereditary autosomal dominant disorder. Patients with LS have an increased risk of cancer development. Estimated risk by the

age of 70y.o. for non-polyposis colorectal cancer (CRC) is up to 82%, endometrial cancer-60%, gastric cancer-13%, etc. LS is caused by mutations in *MLH1, MSH2, MSH6, PMS2, EPCAM* genes. Prevalence of LS is 1:1000. The goal is to define the role of molecular genetic testing and medical genetic counseling in patients with CRC.

Materials and Methods: Case report of 36-year-old male patient with Non-polyposis CRC. The bowel obstruction had occurred in 4 generations of his family. Patient's brother developed CRC at 27y.o., mother-at 49 and at 60y. o. Grandmother and grand-grandfather died of CRC at 62 and 45 years respectively. Meanwhile, proband has 4 children (4-10y.o.). Basing on the clinical signs and pattern of inheritance, LS was assumed. The patient was tested for mutations in LS-associated genes by using target NGS panel.

Results: Sequencing of proband's DNA revealed nonsense mutation p.Arg100Ter in the exon 3 of *MLH1* gene in heterozygous state. The genetic test results proved LS in the proband. Prophylactic genotyping was carried out in all proband's relatives. 3 out of 4 proband's children, brother and most of his children (3 out of 4), sister and mother appeared to be carriers of the same mutation.

Conclusions: Personalized medicine and molecular genetic testing, nowadays, allow to determine precisely the cause of cancer and also to find effective treatment and to develop preventing program for presymptomatic proband's relatives.

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P15.23B
Genomic restricted maximum likelihood (GREML) analysis to estimate the heritability of response/ resistance in major depressive disorder (MDD)

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Introduction: MDD is the leading cause of disability worldwide and lack of response to treatment is reported in ~30% of subjects. Pharmacogenomics studies performed so far failed to identify single SNPs with a replicable effect on anti-depressants response (AD). Instead of focusing on single SNPs, our study aims to evaluate the aggregated contribution on AD response for genome-wide variants and currently known genetic variants from pharmGKB, the main catalog of known pharmacogenetic variations.

Methods: We used GREML-LDMS (LD- and MAF-Stratified) analysis to estimate the heritability of Citalopram response and treatment-resistant status (TRD) in MDD patients from the STAR*D anti-depressants pharmacogenetic trial. We estimated the overall genetic contribution for genome-wide SNPs present in the dataset, as well as for different groups of candidate SNPs from pharmGKB.

Results: We detected a small, but significant, proportion of variance explained by SNPs associated with Citalopram in pharmGKB when including those with the lower level of evidence (V(G)/Vp 0.04; SE 0.02; p 2.23E-05). High heritability emerged when considering genome-wide SNPs for Citalopram response (V(G)/Vp 0.76; SE 0.23; p 0.026), Citalopram symptoms improvement (V(G)/Vp 0.82; SE 0.22; p 0.046) and TRD phenotype (V(G)/Vp 0.81; SE 0.24; p 0.022).

Conclusions: Our preliminary results confirm a little effect of single SNPs and suggest that taking into account the overall genetic variability of pharmGKB AD-related SNPs and, even more, of genome-wide SNPs could improve antidepressant response/resistance prediction.

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P15.24C
Plasma detection of androgen receptor variant 7 in metastatic prostate cancer

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Introduction: Liquid biopsy has the potential to provide information about cancers without invasive biopsy, using circulating biomarkers. In metastatic PCa, progression from a hormonesensitive state to castration resistance under androgen deprivation therapy marks the transition to the lethal phenotype of the disease. Previous studies suggested the presence of AR-V7 transcripts in CTCs, in exosomes and as cell-free RNA in plasma. The aim of the present study was to determinate the presence of Arv-7 in plasma using capillary nano-immunoassay in metastatic PCa and to correlate with clinical and analytical data.

Methods: The study included 31 patients with diagnostic of metastatic PCa. Whole blood (10 mL) was collected in EDTA tubes. Plasma was removed after centrifugation at 1,600 rpm at 4ºC, previously to determinate the presence of ARV-7 in plasma, Albumin and IgG depletion was performed using the Pierce ™ Top 2 columns. Capillary Electrophoresis Immunoassay or SimpleWestern analyses were performed using the WES™ according to the manufacturer's protocol.

Results: AR-V7 signal was detected in 11 cases (36%). Twenty samples (71%) were considered negative. All patients with AR-V7 expression, showed a Gleason score ≥7, higher PSA and was associated with higher values for FA.

Conclusions: Assessing the presence of AR-V7 in plasma from PCa patients is feasible by a novel capillary nano-immunoassay. AR-V7 was observed in 36% of the PCa metastatic patients. These findings lay the foundations for liquid biopsy as a means of obtaining biological data. This study was funded by AECCSal16/002.

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P15.26A

PTEN expression and mutations in *TSC1*, *TSC2* and *MTOR* are associated with response to rapalogs in patients with renal cell carcinoma

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mTOR pathway inhibitors are key drugs for the treatment of many tumor types, however, no predictive biomarkers have been identified so far. Here, we performed a molecular and immunohistochemical characterization of key mTOR pathway components in a series of 113 renal cell carcinoma (RCC) patients treated with everolimus or temsirolimus, aimed at identifying markers of treatment response. Mutational analysis in *MTOR*, *TSC1* and *TSC2* was performed through targeted next-generation sequencing (NGS), and immunohistochemistry (IHC) was performed for PTEN, pAKT, pS6K1, pS6 and p21. Among 89 patients with NGS data, 13.5% had mTOR pathway mutations (9% *MTOR*, 1% *TSC1*, 3% *TSC2*), with the mutation rate decreasing to 8% when only considering pathogenic variants, and with a multiregion NGS analysis in 14 patients giving homogenous results. When comparing the molecular data with the RECIST response of the patients, we found that partial response was more frequent in cases with mTOR pathway mutations than in those without mutations (67% versus 21%, P=0.020). Regarding IHC, negative PTEN staining was detected in 58% of the tumors, and it was more frequent in rapalog responder patients (P=0.024). Mutations and PTEN IHC were not mutually exclusive events and its combination improved response prediction (P=0.013). The staining of other proteins did not show and association with response and no association with PFS was observed for none of the markers analyzed. In conclusion, our study supports mTOR pathway mutations, negative PTEN IHC, and their combination, as markers of rapalog response, proving a step forward the personalization of RCC treatment.

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P15.27B

Comprehensive genetic characterization and evaluation of clinical response in a neurofibromatosis type 2 patient treated with bevacizumab

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Neurofibromatosis type 2 (NF2) is a tumor predisposition syndrome characterized by the development of schwannomas, especially bilateral vestibular schwannomas (VS), and meningiomas. Heterozygous pathogenic variants in the *NF2* gene are known to cause NF2, whereby somatic mosaicism is present in ~25% of simplex patients. In schwannomatosis, a disorder phenotypically similar to NF2, heterozygous *SMARCB1* or *LZTR1* variants may be causative. Recently, bevacizumab has shown efficiency as therapy for VS in some NF2 patients. We report on a thirty-three-year-old patient with bilateral VS, fourteen additional schwannomas and one intracranial meningioma. Next generation sequencing using the TruSight Cancer Panel and Sanger sequencing of *LZTR1* on blood and oral mucosa DNA revealed no pathogenic variants in *NF2*, *SMARCB1* or *LZTR1*. Sanger sequencing on DNA from three schwannomas identified the known nonsense variant c.784C>T;p.(R262*) of *NF2* (NM_000268.3, GRCh37/hg19) in all tumors, leading to the diagnosis of NF2 mosaicism. Because of hearing impairment and tumor progression the patient underwent an off-label therapy with 5mg/kg bevacizumab. Here, we evaluated MRI scans from five pre-therapeutic and two therapeutic years and pure-tone audiometry. After 25 months of treatment, the pure-tone average decreased by 7.5dB, indicating a hearing benefit. Four of seven non-vestibular schwannomas showed volume reduction of ≥20% after 24 months of treatment while the growth rate of the meningioma decreased. In conclusion, in a patient with NF2 somatic mosaicism, an off-label therapy with bevacizumab was efficient with respect to hearing improvement and tumor shrinkage of some non-vestibular schwannomas over a period of two years. (EKFS 2014_Promotionskolleg.22)

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P15.29D
Cardio Diabesity: Could genetics help us to raise awareness of the obesity-related risks?

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One of the leading causes of death in the west world is linked to obesity. The world health organization has estimated that in 2016 over 650 million adults were obese. Those people are more inclined to develop cardiovascular diseases (CVD) and type 2 diabetes (T2D) which are genetic and chronic multifactorial diseases. We performed a genetic screen over 3000 patients tested in 2018 from the Mediterranean area by using the MassARRAY® method that simultaneously analyses 30 genes variations per sample. The genes investigated in our study were previously established to increase the likelihood of developing obesity, CVD and T2D. Three groups of genes were then created from disease-related genes variants with at least one high-risk allele (12 for obesity, 5 for T2D and 4 for CVD). Our finding suggests that risk-alleles frequency for obesity-dependent genes such as *FTO*, *SH2B1* and *ADRB2* have a higher incidence within the tested population with respect to the WT allele. CVD and T2D-related genes such as *MnSOD2* and *TCF7L2* showed a similar incidence. We additionally find that 65% of tested samples for FTO high-risk allele had at least one high-risk allele for either or both *MnSOD2* and *TCF7L2*. Thus, people genetically predisposed to develop obesity can be also inclined to develop CVD or/and T2D. The incidence of those diseases could be decreased in the future by combining the genetic screens with balanced diets and physical activity. Specifically, the genetic screen can raise awareness of developing deadly diseases especially in high-risk people.

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P15.31B
Genetic polymorphisms in dopaminergic pathway influence the occurrence of adverse events of dopaminergic treatment in Parkinson's disease

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Introduction: Defects in dopaminergic pathway are the characteristic underlying processes of Parkinson's disease (PD). Genetic variability in this pathway may influence the outcome of dopaminergic treatment (DT), especially in terms of adverse events (AEs). We have used pathway-based approach to comprehensively evaluate the influence of selected single nucleotide polymorphisms (SNPs) of key dopaminergic pathway genes on the occurrence of AEs of DT in PD.

Material and Methods: We recruited 231 PD patients on DT and collected data on their AEs: sleep attacks, visual hallucinations, nausea/vomiting, orthostatic hypotension, peripheral oedema, impulse control disorders, motor fluctuations and dyskinesia. We genotyped patients for the following SNPs: *COMT* rs4680, rs165815, *DDC* rs921451, rs3837091, *MAOB* rs1799836, *DRD2* rs1801028, rs1799732, *DRD3* rs6280, *SLC6A3* rsrs393795, rs6347,

rs104209, *SLC22A1* rs628031, *SLC18A2* rs14240, and *SV2C* rs1423099. In logistic regression analysis, results were adjusted for significant clinical parameters.

Results: *COMT* rs165815 C allele decreased odds for visual hallucinations (OR=0.34, 95%CI=0.16-0.72, p=0.004), while *DRD3* rs6280 CC genotype increased the risk for this AE (OR=3.31, 95%CI=1.37-8.03, p=0.008). *DDC* rs921451 CT genotype, *DDC* rs3837091 AGAGdel genotype, and *SLC22A1* rs628031 AA genotype increased odds for orthostatic hypotension (OR=2.30, 95%CI=1.26-4.20, p=0.007; OR=1.94, 95%CI=1.07-3.51, p=0.028, OR=2.57, 95%CI=1.11-5.95, p=0.028, respectively). *SLC22A1* rs628031 AA genotype increased odds for peripheral oedema and impulse control disorders (OR=4.00, 95%CI=1.62-9.88, p=0.003, OR=3.16, 95%CI=1.03-9.72, p=0.045, respectively). *SLC22A1* rs628031 GA genotype protected patients against dyskinesia (OR=0.48, 95% CI=0.24-0.98, p=0.043).

Conclusion: We have identified a few new potential predictive genetic biomarkers of AEs of DT in PD.

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P15.32C
Allele and genotype frequencies of pharmacogenomic markers in a latino population: findings from the Latin American Personalized Medicine Association

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Introduction: Latin Americans represent one of the largest admixed populations in the world. They also represent ~18% of the total US population[1]. While pharmacogenomic (PGx) markers have been well characterized for certain ethnicities, little has been published on the frequency of relevant PGx genotype and phenotype rates in latinos.

Materials and Methods: Attendees of the 2018 ALAMP conference were asked to participate in this study and subsequently consented to receiving pharmacogenomic testing using the RightMed® Comprehensive test (OneOme, USA). 146 predominantly latino participants completed the study. Participants were tested for 27 pharmacogenes and received genotype-derived recommendations for more than 360 medications as well as a genotype and phenotype summaries. Medications on each report were binned into red (major drug-gene interactions), yellow (moderate) and green (minimal).

Results: Participants averaged 8 red medications (major interaction), and every participant had at least 1 major or moderate drug-gene interaction identified. Of interest, 20% of participants were intermediate metabolizers of clopidogrel, indicating a loss of function allele in CYP2C19. In addition, 34% carried the SLC6A4 S/S genotype, associated with lowered tolerability of SSRIs, and 45% were carriers of UGT1A1 variants, posing a severe risk of neutropenia and toxicity for those with certain chemotherapy regimens.

Conclusion: For PGx to become standard of care in medicine, it is imperative that different ethnic groups be represented in the literature and ultimately, in professional guidelines. Through this study, we have made strides to characterize the latino pharmacogenomic profile, which will in turn help to stratify patients for PGx testing.

B. Esquivel: A. Employment (full or part-time); Significant; Oneome. **T. Koep:** A. Employment (full or part-time); Significant; OneOme. **M. Donaldson:** A. Employment (full or part-time); Significant; OneOme.

P15.33D
Implementational insights from AZ/Medimmune's Genomics Initiative

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Introduction: In 2016 AstraZeneca launched a company-wide genomics initiative aiming to integrate genomics throughout the drug discovery and development pipelines. The initiative will analyse up to 2 million genomes, including sequencing of 500,000 patients participating in company sponsored clinical trials.

To achieve this, novel processes and documentation had to be rapidly embedded across therapeutic areas, replacing restricted, study specific pharmacogenetic samples with broadly consented genomic samples allowing use across health-related research.

Methods: Three steps were employed to enable this strategy: 1) a novel role was created to operationally support study teams and monitor implementation; 2) a dedicated mailbox was set-up as a primary point of contact for genomics queries and advice; 3) training material, face to face "working groups" and training sessions were rolled-out across the organisation. To assess the impact of these measures, the inclusion of genomic sample collection across clinical studies was audited.

Results: During 2018 over 1400 email items, addressing queries from 53 different study teams were received and resolved in the genomics mailbox and 13 formal training sessions were held with more than 250 attendees from multiple company functions including clinical teams,

operational leads, data managers and process owners. Implementation of these measures resulted in 150% increase in the number of studies collecting genomic samples (60% of all AZ studies) with over 14,000 patients approached.

Conclusion: A genomics initiative, collecting broadly consented samples, can be successfully embedded within a global organisation by dedicating resources that raise awareness and provide a robust support structure for study teams.

H.E. Stevens: A. Employment (full or part-time); Significant; Astrazeneca.

P15.35B

Implementing pharmacogenetics by repurposing next generation sequencing data on a personalized card

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Introduction: Pharmacogenetics is one of the first clinical applications of personalized medicine. However, in current clinical practice it is often used retrospectively, while patients may benefit most from preemptive testing [1]. Increasing use of Next Generation Sequencing offers new opportunities for large scale implementation of pharmacogenetics in clinical practice.

Materials and Methods: Whole Exome Sequencing (WES) for children and their parents is routinely performed for diagnosis of certain medical conditions. We created a post-processing procedure that phases all variants based on trio analysis. The pharmacogenetics profile is extracted from approximately 200 phased variants in 12 genes. Patients are offered a personal card with a QR code that contains the profile. On access of their personal page, the drug metabolizer phenotype is predicted, and individual drug recommendations are generated based on the monthly updated national drug database *G-Standaard* [2].

Results: In this multicenter project we developed a system that reuses diagnostic WES data for pharmacogenetics. The systems consists of a platform to deliver pharmacogenetic profiles extracted from diagnostic WES data, together with a website that provides personalized drug recommendations established by the Dutch Pharmacogenetics Working Group. Preliminary analysis yields pharmacogenetic recommendations for 1321 out of 1845 profiles (72%).

Conclusions: Our system allows for large scale implementation of clinical pharmacogenetics for patients that undergo WES. The recommendation system is agnostic of the genotyping method and designed to work with other methods.

References

[1] "Benefit of Preemptive Pharmacogenetic Information on Clinical Outcome", Roden et. al., Clinical Pharmacology & Therapeutics, 2018

[2] https://www.z-index.nl/english

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P15.37D

TNFAIP2 upregulation and *VAMP8* downregulation may be genetic biomarkers of metabolic Adverse Drug Reactions of antipsychotic treatments as detected by RNA-seq expression analysis on a healthy volunteers' study

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Introduction: Schizophrenia affects up to 2% of the European population. Being a chronic and disabling disorder, it requires lifelong medication, that subsequently triggers metabolic Adverse Drug Reactions (ADRs). This project aims to identify novel biomarkers that may contribute to the short-term prediction of metabolic dysfunctions associated with antipsychotic treatments.

Materials and Methods: A clinical trial with healthy volunteers was carried out to evaluate the short-term effect (5 days) of olanzapine and aripiprazole. Each volunteer received both treatments separated by one month of washout. Blood samples were collected before and after the treatment. A total of 16 samples were analysed with

Illumina total RNA-Seq technology, followed by a differential expression analysis.

Results: Gene Set Enrichment Analysis (GSEA) showed that aripiprazole upregulated TNFα/NFKB signalling ($p < 0.001$), that controls inflammatory response, which also became upregulated ($p < 0.05$). Chronic inflammation is associated with obesity, a feature of the metabolic syndrome. *TNFAIP2* that is regulated by proinflammatory molecules, increased its expression with aripiprazole ($p = 0.0001$, Wald test). Olanzapine treatment repressed SNARE proteins ($p < 0.005$), that facilitates glucose uptake, which disruption is also affected by insulin resistance. Among them, *VAMP8* was the most downregulated gene ($p = 0.01$, Wald test). Other validations are ongoing.

Conclusions: Our results suggest that aripiprazole could be related to the upregulation of chronic inflammatory response, while olanzapine may induce insulin resistance through SNARE proteins repression. *TNFAIP2* and *VAMP8* could be candidate biomarkers for the short-term prediction of metabolic ADRs. This project was funded by the EU Framework Horizon 2020 under GA 721236.

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P15.39B
Genomics as a personalized medicine approach in disease risk prediction - P5.fi FinHealth

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Introduction: In P5.fi we utilize polygenic risk scores to provide personalized information on the individual disease risk related to three common diseases (coronary heart disease, type 2 diabetes and venous thromboembolism) for 3.400 volunteering participants. We hypothesize that genetic risk information would improve prevention, diagnosis and treatment.

Materials and Methods: We validated the polygenic risk scores in whole genome genotyped population based FINRISK cohorts (N=20.000) using Cox regression models. Follow up data from national health care registers allowed us to model the impact of genetic and traditional risk factors such as smoking, cholesterol, blood pressure and BMI on a person's risk of disease within the next 10 years.

Results: We observed that type 2 diabetes (T2DM) PRS significantly associates with the T2DM disease risk (HR:1.5 per 1 sd PRS, p-value:$<2*10^{-16}$). Also the top 8% of the FINRISK population who had inherited the highest PRS had fourfold increased risk for T2DM. Moreover, people with >35 BMI and the highest PRS tend to get diabetes at younger age.

Conclusions: FINRISK cohorts produced estimates on the impact of PRS and selected covariates on risk of T2DM. We use these estimates to assess the future risk of T2DM in P5.fi FinHealth participants. Participants will receive this disease risk information including genetic risk via a web portal. We will monitor the reception of the information by questionnaires and follow the participants for disease end points using registry data.

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P15.40C
Partial polygenic scores for individuals of mixed ancestries

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Polygenic Scores (PS) are a combined measure describing an individual's genetic component for a quantitative phenotype or his susceptibility for a disease with a genetic basis. PS rely on contributions of many associated alleles extrapolated from GWAS, which are partly population-dependent. This poses serious limitations to the applicability of PS to admixed individuals, where at least one of the mixing ancestry comes from an understudied population. In this case, different portions of the genome should be treated differently, to account for the population-specific nature of the association signals.

Here we introduce Ancestry Specific Partial-PS, a novel approach that computes partial PS on ancestry-specific chromosomal segments, obtained through local ancestry deconvolution. We first assess what are the effects of taking only a fraction of the genome to compute a PS, how to take into account the uncertainty deriving from the missing part,

and Partial-PS behavior across populations. Then we apply this method on populations with admixed African and West Eurasian ancestry where, with a standard whole genome approach, no reliable PS can be computed for the reasons mentioned above. Using Partial-PS we could retrieve in simulated individuals part of the total PS predictivity in proportion to the European ancestry fraction and up to 80% in modern Egyptians, whose West Eurasian component is close to 80%. These results may inform the applicability of PS to subjects that are currently left out of the personalized medicine revolution due to their complex, individual history of admixture.

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P15.41D
Identifying genetic variants associated with ritodrine induced pulmonary edema

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Introduction: Ritodrine is one of the most commonly used tocolytics in preterm labor, as a ß2-adrenergic agonist which reduces intracellular calcium levels and prevents myometrial activation. Ridotrine infusion can result in serious maternal complication, and pulmonary edema is a special concern among the complications. The cause of pulmonary edema following ritodrine treatment is multifactorial, but the contributing genetic factors remain understudied. This study investigates the genetic variants associated with ritodrine induced pulmonary edema.

Materials and Methods: In this case-control study, 16 patients who developed pulmonary edema during ritodrine infusion [case] and 16 pregnant women who were treated with ritodrine but did not develop pulmonary edema [control] were included. Control pregnant women were selected after matching for plurality and gestational age at use of tocolytics. Maternal blood was taken at the time of admission for tocolytics, and whole exome sequencing was performed with stored blood samples.

Results: A total of 71 candidate genes were selected by comparing the cumulative effects of multiple coding variants between patients with pulmonary edema and matched controls for 19729 protein-coding genes. Subsequent data analysis selected only statistically significant and deleterious variants with a role compatible with ritodrine-induced pulmonary edema. Two final candidate variants located in CPT2 (carnitine palmitoyltransferase 2) and ADRA1A (Alpha-1A adrenergic receptor) were selected.

Conclusions: We identified new potential variants in genes that play a role in cAMP/PKA regulation, which supports their putative involvement in predisposition to ritodrine-induced pulmonary edema in pregnant women.

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P15.42A
Association between self-rated general health and telomere length may be modified by genetic variants associated with telomere length

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Introduction: Self-rated general health is an important predictor of various aging-related adverse health outcomes such as comorbidities, mortality, and healthcare utilization. Telomere length (TL) is a biomarker of accelerated aging. We aimed to assess whether TL is associated with self-rated general heath and whether this association differs by genetic predisposition to short TL.

Materials and Methods: Peripheral blood leukocyte samples from 99 subjects enrolled in the Mayo Clinic Biobank were used to measure TL and three TL-associated SNPs (rs7726159 [TERT]; rs1317082 [TERC], and rs2487999 [OBFC1]). Self-rated general health (excellent, very good, good, and fair/poor) was collected using a questionnaire. Adjusting for age and sex, linear regression models were used to test association of TL with self-rated general health and its interaction with the three SNPs.

Results: The cohort was relatively young (median age: 45 years) with 65% females and 100% Whites. The median TL was 1.40 (25^{th}-75^{th} percentiles: 1.39-1.42). TL decreased with worse self-rated health in a dose-response manner (e.g., 0.017 shorter TL in poor/fair health vs. excellent health; p=0.002). The association may be modified by TL-associated SNPs (interaction p=0.001 with rs7726159, and 0.006 with rs287999).

Conclusions: Our pilot study demonstrates that TL is associated with self-rated general health, implying that TL may serve as a biomarker for self-rated general health. Our study also suggests that this association may differ by genetic predisposition for shorter TL. Given that this study lacks statistical power due to a small sample size, further investigation of these findings in a larger sample is encouraged.

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P15.43B

Diagnosis of over one third of heterogeneous rare disease cases from the BBMRI-LPC 800 WES call using the RD-Connect Genome-Phenome Analysis Platform

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We report the outcome of the BBMRI-LPC WES Call, undertaken in collaboration with RD-Connect (https://rd-connect.eu/) and EuroBioBank (http://www.eurobiobank.org/). Over 800 whole exomes were sequenced free-of-charge in seventeen distinct projects, each focusing on a particular rare disease and involving PIs from at least two different countries. A requirement of the call was that a specimen from each case be deposited within the EuroBioBank network, to be available to the wider rare disease research community. Phenotypic information for each affected case was collated with PhenoTips using standards such as the Human Phenotype Ontology (HPO), OMIM and Orpha codes. Variant filtration and prioritisation was undertaken using the RD-Connect Genome-Phenome Analysis Platform (GPAP, https://platform.rd-connect.eu).

All samples were processed using the RD-Connect standard analysis pipeline (Laurie et al, Human Mutation, 2016), and reliable variants uploaded into the GPAP. Analysis and interpretation of cases was undertaken by a clinical genomics specialist and relevant disease-domain experts. Advanced GPAP tools such as on-the-fly gene-lists based upon gene-HPO relationships, Exomiser, homozygosity mapping, pathway associations, and matchmaking helped facilitate causative variant identification.

Despite the heterogeneous nature of the different phenotypes under investigation e.g. neuromuscular disorders, albinism, inborn errors of metabolism, the molecular diagnostic rate was over 33% overall. This is in line with similar studies which have focussed on a more restricted range of phenotypes. Furthermore, ten novel gene-disease relationships are currently undergoing functional validation, and we anticipate more cases will be resolved through inclusion in the SolveRD project (http://solve-rd.eu/).

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P15.44C

Diagnosis of neurological rare disease patients through genomic reanalysis within the URD-Cat project on personalised medicine

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The Undiagnosed Rare Disease Program of Catalonia (URD-Cat) is a pilot project aiming to facilitate implementation of personalised medicine for rare diseases within the Catalan Health System, focusing on rare neurological conditions as a use case. Genotypic and phenotypic data is collated, integrated and analysed using the URD-Cat platform, based on the RD-Connect Genome-Phenome Analysis Platform. The URD-Cat platform integrates previously available and newly generated clinical and genomic data from 1,454 individuals (835 probands).

Two-hundred and forty-eight probands (421 individuals including relatives) for whom genomic data was available in 7 hospitals across Catalonia were reanalysed between 2-8 years after initial data generation (mean 4 years). Standardised phenotypic and clinical information were collected using the Human Phenotype Ontology (HPO) and the Orphanet Rare Disease Ontology (ORDO). Genomic data (63 gene panels, 356 whole exomes and 2 whole genomes) were processed with the URD-Cat pipeline, including detection of Copy Number Variants (CNVs) and runs of homozygosity (RoHs). Clinical and genomic data were shared and reanalysed through the URD-Cat platform.

Reanalysis of genomic data revealed causative mutations in 9.7% of probands (24/248) and candidate pathogenic variants in 4.4% (11/248). Most new diagnoses were due to novel gene-disease associations (n=8), technical improvements in variant calling (n=3), detection of CNVs (n=2) and RoHs (n=1), and URD-Cat platform functionalities such as filter by HPO-related genes or pathogenic variant prioritisation (n=2). Identification of causative variants also enabled the diagnoses of three newly sequenced cases thanks to internal patient matchmaking enabled by sharing data across the network.

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P15.45D

Genotype first female breast cancer risk-based screening and management, initial lessons from a national pilot project

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Breast cancer screening and management will move from age- to risk-based management. The genetic component of breast cancer risk originates from monogenic variants, as well as polygenic risk. In 2018, Estonian Biobank, University of Tartu, Estonia, together with two central hospitals in Estonia, Tartu University Hospital and North Estonia Medical Center have initiated a pilot study for genetics-based breast cancer screening and follow-up management in female participants of the biobank.

The study consists of monogenic and polygenic arms. We aim to estimate the possible impact of genetic risk for breast cancer prevention and early detection, as well as to test the subject compliance, psychosocial aspects, data transfer logistics from research to clinical setting, etc. In monogenic arm, cascade screening of relatives will maximise the impact of existing genetic data. The study group consists of female participates 22-74 years. In monogenic arm, 128 subjects with class 4 and 5 variants in 11 genes recurrently associated with moderate to high risk of breast cancer were identified. The clinical follow-up is based on adapted international guidelines. In polygenic arm, the participants with top 5% threshold were selected as high-risk group (HR 2.73, 95% CI 1.92-3.9). The females (n ~1300) will receive information about their genetic risk and biennial mammography will be initiated from age 40.

We will present the data on study progress and compliance, as well as discuss the ethical and communication lessons learned, along with perspective on public healthcare.

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P15.46A

Importance of SLC6A4 promoter Short and Long Allelle in predisposition and in determining the optimal drug dosage in patients with majör depression

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Introduction: Serotonin (5-hydroxytryptamine, 5-HT) is synthesized in serotonergic neurons, from tryptophan. SLC6A4 is the gene that codes for the serotonin transporter (5-HTT), responsible of reuptaking 5-HT. Functional insertion / deletion polymorphism found in the gene promoter region (5-HTTLPR) primarily effects 5-HT metabolism; short (S) allele has been reported to be associated with less transcriptional efficiency compared to allele with long (L) allele. Aim of the study is to investigate the distribution of L and S allelles in patients with major depression.

Materials and Methods: 49 patients were enrolled for the study. All patients were informed about the study protocol, each patient received written approval prior to the registration. Conventional PCR methodology were carried out for the genotyping of 44 bp S/L allelles.

Results: The numbers of the patients and their percentages were 7 (14%), 26 (53%) and 16 (33%) for the genotypes LL, LS and SS, respectively. Allelic count gave rise to 40 (41%) and 58 (59%) for L and S allele, respectively.

Conclusion: Determining the genotype of patients with depression is important for treatment and also for the appropriate dose of anti-depressant SSRIs drugs. In addition, the L allele with normal metabolic function compared to the S allele of SLC6A4 is less common in depressed individuals. Having knowledge about the genotype of SLC6A4 will encourage both individuals and clinicians to have a life style protecting against depression. But studies with larger cohorts are needed to fulfill the role of this polymorphism on the onset of depression.

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P15.47B

Prediction of nephrotoxicity associated with cisplatin-based chemotherapy in testicular cancer patients

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Introduction: In industrialized countries, testicular cancer (TC) is the most common solid tumor in men between 20-40 years old. Besides being one of the most treatable types of cancer, the long-term side-effects of chemotherapy, such as nephrotoxicity, are worrisome, since they are largely irreversible. The standard treatment for TC is 3 cycles of cisplatin, etoposide and bleomycin (BEP). Here, we focus on nephrotoxicity, measured as the drop in glomerular filtration rate after chemotherapy.

Materials and Methods: Clinical patient data on 420 individuals was collected from hospital files, and saliva samples were used for genotyping using Illumina® HumanOmniExpressExome-8-v1-2-B-b37 chip (nearly 1 million markers).

Machine learning (ML) models (random forests) including clinical and genomic features were built for classifying patients at risk of developing nephrotoxicity given a treatment of BEP-cycles.

Results: First, only clinical features, such as age at the time of treatment, dose of cisplatin, patient's prognosis, and number of cycles, were considered for training the model, and relevant features were selected (based on Gini coefficient) to use in the classifier (AUC 0.66 – sensitivity 0.59, specificity 0.61). The classifier was then re-trained by adding genomic markers. An AUC of 0.75 (sensitivity 0.66, specificity 0.67) was obtained through ML combination clinical and genomic features for nephrotoxicity prediction in patients receiving cisplatin-based chemotherapy. The presentation will include the top clinical and GWAS SNPs.

Conclusions: The study proposes a ML algorithm which, by helping predicting nephrotoxicity in advance, can improve treatment efficacy in TC patients by allowing a more personalized treatment to each patient.

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P15.48C

Automated classification of ~7,000 variants shows near perfect concordance with expert panel assessments

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Introduction: Gathering the most current and accurate information is critical to variant interpretation. The QIAGEN knowledgebase includes a manually curated database of variant specific publications, and data from public and proprietary databases. This resource is the cornerstone of QIAGEN Clinical Insight (QCI), which facilitates automated variant classification using all 28 ACMG rules, while transparently providing the underlying evidence. Here, we compare the concordance of QCI's automated variant classification with expert panel assessments across multiple disease indications.

Materials and Methods: Expert panel (ENIGMA) reviewed *BRCA1* and *BRCA2* variants (n=6154), and variants reviewed by the ClinGen Inherited Cardiomyopathy Expert Panel (n=102) were exported from ClinVar. Resulting VCFs were uploaded into QCI, and concordance of automated classifications was compared with expert panel assessments in ClinVar.

Results: With respect to clinical actionability, automated variant classifications were extremely concordant, reaching 99.6% concordance with ENIGMA assessments of *BRCA* variants, and 96.1% concordance with cardiomyopathy variants assessed by the ClinGen expert panel. The small number of differences seen in the ENIGMA dataset could be attributed to functional studies used by the automated algorithm not considered by the expert panel. The 3.9% of discrepant classifications in the cardiomyopathy set likely result from differences in clinical case curation.

Conclusions: Through updated content, and continued alignment with professional guidelines, automated variant classification in QCI demonstrates extremely high accuracy across multiple disease contexts. This level of accuracy speaks to the quality of the clinical, functional, and population level data curation, as well as the robustness of the underlying ACMG classification algorithm.

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P15.49D
How good are variant pathogenicity predictors?

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Computational tools are widely used for interpreting variants detected in sequencing projects. The choice of these tools is critical for reliable variant impact interpretation for precision medicine and should be based on systematic performance assessment. The performance of the methods varies widely in different performance assessments, for example due to the contents and sizes of test datasets. To address this issue, we have collected benchmark datasets to VariBench (http://structure.bmc.lu.se/VariBench/) and VariSNP (http://structure.bmc.lu.se/VariSNP/) and performed performance assessments for disease-causing and benign variants, and variants in different regions in membrane proteins. Recently we investigated 63,160 common amino acid substitutions (allele frequency >1% and <25%) in ExAC database to determine the specificity for 10 variant interpretation tools. In addition to overall specificity of the tools, we tested their performance for variants in six geographical populations. PON-P2 had the best performance followed by FATHMM and VEST. While these tools had excellent performance, the poorest method predicted more than one third of the benign variants to be disease-causing. The results allow choosing reliable methods for benign variant interpretation, for both research and clinical purposes, as well as provide a benchmark for method developers. We have developed tools also for the mechanisms of variants including protein stability, solubility, steric clashes, disorder etc. Financial support: Swedish Research Council References: Niroula, A. and Vihinen, M. How good are pathogenicity predictors in detecting benign variants? PLoS Comput. Biol. (in press). Orioli, T. and Vihinen, M. Benchmarking membrane proteins: Subcellular localization and variant tolerance predictors. (revised).

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P16
Omics - Bioinformatics

P16.01A
Opioid, dopamine, and GABA receptor addiction and mental health enriched genomic areas co-located with 16 cancer, immunity and Parkinson's disease drug binding sites

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The adverse impact of opiate, dopamine, and alcohol addiction on human populations is well known and exacerbated by mental health co-morbidities including schizophrenia, bipolar disorder, and depression. These disorders have deep genomic roots that modulate disease severity, duration, and potential therapeutic outcomes. Our previous work demonstrates that using an *in silico* functional genomics and evolutionary biology framework can identify functionally relevant enriched genomic hotspots and variants with import for opioid, dopamine and alcohol addiction complex polygenic traits. This study seeks to extend this approach to determine its utility in identifying

pharmacogenomics therapeutic targets at addiction and mental health hotspots. To evaluate whether the intersection of NCBI curated genes identified as participating in addiction phenotypes along with schizophrenia, bipolar disorder and depression (N=1968 genes), genes lists were projected onto the human genome to find eight areas of addiction and mental health gene set enrichment. Functional annotations were conducted for core phenotype functions. Finally, drug binding annotations were conducted to determine whether the binding sites sitting in these addiction and mental health hotspots were related to the functional or gene set enrichment. We found that 16 pharmacogenomic drug binding sites were identified in this analysis which broadly fell into four categories: cancer, addiction, immunity and mental health related drug effects. The cancer drug target all had addiction, loss of immune robustness, and anxiety as their side effects. These findings suggest that this *in silico* approach can potentially inform drug target effects and may be useful in informing novel and drug-repurposing pipelines.

L. Jackson: None.

P16.02B
Genetic dysregulation of gene expression and splicing during a ten-year period of human aging

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Molecular and cellular changes are intrinsic to aging and age-related diseases. Prior cross-sectional studies have investigated the combined effects of age and genetics on gene expression and alternative splicing; however, there has been no long-term, longitudinal characterization of these molecular changes, especially in older age. We performed RNA sequencing in whole-blood from 65 healthy participants from the Prospective Investigation of Uppsala Seniors study at both age 70 and 80, a period of the aging process characterized by high morbidity and mortality. We observed that 93% of individuals are more similar to their own expression profiles later in life than profiles of other individuals their own age. We identified 1,291 and 294 genes differentially expressed and alternatively spliced with age, as well as 529 genes with outlying individual trajectories. Further, 7.8% and 9.6% of tested genes show a reduction in genetic associations with expression and splicing in older age, with impacted genes enriched in DNA repair pathways. In addition, we observed a high genetic correlation between ages ($\rho_G = 0.96$). In contrast, overall allelic imbalance within an individual increases with age by 2.69%. These findings demonstrate that, although the transcriptome and its genetic regulation is mostly stable late in life, a small subset of genes is dynamic and is characterized by reduction in genetic regulation. The strong correlation of genetic effects and the increase in allelic imbalance with age demonstrates that increasing environmental variance, as opposed to decreased genetic variance, underlies the reduction in genetic regulation with age.

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P16.05A
CRISPY: a PYthon module for the analysis of gene editing experiments by CRISPR

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Dedicated bioinformatic tools are essential to evaluate the accuracy of CRISPR-Cas9 gene editing. Most of the web-based algorithms, such as Cas-Analyzer, focus on the frequency of correction rather than providing a detailed outcome of the experiment. On the other hand, command line tools usually provide more details, but they are not easily accessible to researchers that are not experts in bioinformatics. In order to combine detailed outcomes with easy-to-use, we implemented a self-contained python module with a graphical user interface for the analysis of gene editing experiments. CRISPY first performs the alignment against the reference genome starting from raw NGS reads, and then it considers all the experimental sequences overlapping the region of interest, regardless of their lengths, without any sort of filtering. CRISPY assesses the editing accuracy by determining the amount of successfully edited sequences in the treated samples with respect to the control experiment, classifying as edited only

the identical sequences without mutations, therefore indicating the true rate of Homology Directed Repair. It is able not only to evaluate the rate of Non Homologous End Joining, by computing the indel frequency but also to assess the complessive biological efficacy counting separately synonymous and nonsynonymous substitutions induced in the range of interest. From a clinical point of view, edited alleles containing unsolicited synonymous substitutions contribute positively to the correction and it may be worth to be identified and counted. In conclusion, CRISPY is a PYthon based, easy-to-use but raffinate tool for accurate analysis of CRISPR induced gene editing.

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P16.06B
Germline variants classification using bioinformatic tools for next generation sequencing-based classification

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Currently, genetic medicine is increasing in complexity, mainly due to the clinical implementation of next-generation sequencing (NGS). Genetic variants can be associated with unknown and/or complex phenotypes. Missense variants regularly tend to be classified as variants of unknown significance (VUS) leading to inconclusive diagnostic findings. To meet the challenge of variant classification, we developed two bioinformatic tools: one supporting BRCA1/2 variants classification and one supporting the classification of variants found in Mendelian disorders. The variant interpretation relies on two independent classification methods: Consensus and ACMG classification based on public and proprietary data sources. To pre-validate the classification methods, we compared our BRCA tool results with classifications provided by three published Japanese hereditary breast and ovarian cancer studies. The results of the automated, ACMG-guided conservative interpretation of BRCA1/2 variants are comparable to the referenced clinical classification of pathogenicity. Of 108 pathogenic variants, BRCA tool classified 80 as pathogenic, 26 as likely pathogenic, and 2 as VUS. Whereas 11/26 benign variants were classified as VUS, a discrepancy due to lack of public population-frequency and patient-family history data. For the Mendelian tool, we compared the consensus classification with a study, in which 98 germline variants spread over 95 genes with a large phenotypic spectrum were classified. The consensus method classified 59/98 as pathogenic and 7/98 as likely pathogenic, a result identical to the reference classification. Variant classifications were found to be accurately associated to the corresponding hereditary disease. Further refinement of the methodology in close collaboration with laboratory partners is in progress.

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P16.07C
Deciphering exome sequencing data: bringing mitochondrial DNA variants to light

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Introduction: Mitochondrial DNA (mtDNA) mutations are responsible of various phenotypes, causing partial or entirely unexplained phenotypes concerning one or several organs. When suspected, mtDNA variants identification requires targeted strategies on affected tissues. The expanding use of exome sequencing (ES) in diagnosis generates a huge amount of data, including untargeted mtDNA sequences. We thus developed a bioinformatics pipeline on ES data in order to detect mitochondrial variants in parallel with the routinely used "in-house" nuclear exome pipeline, in particular in patients with no initial clinical suspicion of mitochondrial disorders.

Methodology: Mitochondrial DNA data coming from off-target sequences (indirect sequencing) were extracted from the BAM files in 928 individuals with developmental (90%) and/or neurological anomalies (10%). After merging, the variants were filtered out based on database presence, cohort frequencies, haplogroups and protein consequences. In parallel, some patients with previously identified mitochondrial variants were sequenced and tested as positive controls.

Results: Two homoplasmic pathogenic variants (m.9035T>C and m.11778G>A) were identified in 2/928 unrelated individuals (0.2%): the m.9035T>C (*MT-ATP6*) variant in a female with ataxia and the m.11778G>A (*MT-ND4*) variant in a male with a complex mosaic disorder and an unusual severe ophthalmological phenotype, uncovering undiagnosed Leber's hereditary optic neuropathy (LHON). Seven secondary findings were also found, predisposing to deafness or LHON, in 7/928 individuals (0.75%).

Conclusion: This study demonstrates the usefulness of including a targeted analysis strategy in ES pipeline to detect mtDNA variants, improving results in diagnosis and research, without resampling patients and performing targeted mtDNA strategies.

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P16.08D
Gabriella Miller Kids First Data Resource Center: Harmonizing genomic and clinical information to support childhood cancer and structural birth defect research

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Introduction: Childhood cancers and structural birth defects present a huge burden to families as well as the healthcare system. These diseases share a common context of altered developmental biology, but the potential role of genetic alterations and/or pathways across them remains to be investigated.

Materials and Methods: The NIH Common Fund Gabriella Miller Kids First Program represents a national collaborative initiative focused on large-scale genomic and clinical data sharing for childhood cancers and structural birth defects. As part of this program, the Kids First Data Resource Center (DRC) is charged with empowering collaborative discovery across Kids First datasets. Through newly developed platforms and cloud-based resources, researchers are able to access standardized genomic and clinical data in a timely fashion.

Results: Nearly 30,000 genomic and phenotypic files generated for over 9,100 individuals from ~1,700 families are available now at DRC portal, making DRC one of the largest pediatric data resources across a wide range of diseases. Cloud-based computing has greatly facilitated large-scale genomic harmonization and DRC is capable of running 200 workflows simultaneously with considerable scalability on demand. Additionally, there is a strong focus on harmonizing and structuring clinical data to make them more interoperable, discoverable and reusable by using ontologies such as Human Phenotype Ontology.

Conclusions: The combination of harmonized genomic and clinical data across pediatric cancers and structural birth defects provides a key foundation for exploring and

developing new methods to better understand the relationships between germline variants, cancer risk, and associated treatments and outcomes.

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P16.09A
Identification of bacteriophage-related sequences in carcinoma patients and NIPT data of pregnant women

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Introduction: NIPT is ranked among most widespread genomic test in the whole world with approx. 4 million tests carried out in 2017. On the other hand, bacteriophages are the most widespread entities in the biosphere. In our previous work we tried to find out phage-related sequences in standard NIPT data with 2x35bp sequence reads. Such NIPT data has been shown inefficient for a contig assembly or blast. In this study, we mined phage sequence from genomic data of carcinoma patients and longer unmapped NIPT reads of average length 2x100bp.

Materials and Methods: We sequenced total DNA from plasma of healthy people. We assessed presence of phage-related sequences by different bioinformatic approaches. At first, we removed human sequences by mapping to hg38, then we used tools based on k-mers identification, contig mapping and gene-centric approaches, those utilizing our custom phage database. Same methods were used to analyze blood samples from patients with solid tumor.

Results: We detected the presence of phage-related sequences and assessed disparities of aforementioned sets of samples. For instance, we identified sequences belonging to Proteus phage, Escherichia phage PhiX174, Campylobacter phage B14.

Conclusions: NIPT data are like mines, containing unbounded amount of unrevealed informations. Various bioinformatic approaches can be used like mattock to obtain additional results. Our results demonstrate the detection of phage sequences in the NIPT data which are usually marked as useless in common NIPT analyses.

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P16.10B
Sensitive detection of low allele fraction structural variants in clinical cancer samples

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Tumors are often comprised of heterogeneous populations of cells, with certain cancer-driving mutations at low allele fractions in early stages of cancer development. Effective detection of such variants is critical for diagnosis and targeted treatment. However, typical short sequence reads are limited in their ability to span across repetitive regions of the genome and to facilitate structural variant (SV) analysis. Based on specific labeling and mapping of ultra-high molecular weight (UHMW) DNA, we developed a single-molecule platform that has the potential to detect disease-relevant SVs and give a high-resolution view of tumor heterogeneity.

We have developed a pipeline that effectively detects structural variants at low allele fractions. It includes single-molecule based SV calling and fractional copy number analysis. Preliminary analyses using simulated data and well-characterized cancer samples showed high sensitivity for variants of different types at as low as 5% allele fractions with reasonable genomic coverage easily collectable on a Bionano Saphyr Chip. The candidate variants are then annotated and further prioritized based on control data and publically available annotations. The data are imported into a graphical user interface tool that includes new visualization features (such as Circos diagrams) for interactive visualization and curation. The streamlined pipeline is fully compatible with the new Direct Label and Stain (DLS) chemistry. Together, these components allow for efficient analysis of any cancer genome of interest.

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P16.11C

Bioinformatics approach to finding new drug targets in immunological synapse using gene expression as biomarkers and unsupervised machine learning - a comparative study of immune response regulatory networks in subtypes of kidney carcinoma

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Cancer immunotherapy, which utilizes immune system to selectively destroy cancer cells, becomes a safer and more effective alternative for cancer patients' treatment than traditional approaches. However, success rate is low: many patients do not respond to treatment, whereas others suffer from adverse effects.

The immune response is mediated by immunological synapse – an interface between lymphocytes and antigen presenting cells, which consists of co-inhibitory and co-stimulatory checkpoint proteins. Understanding interactions within immunological synapse is crucial for better design of clinical trials and will provide better diagnostics and therapeutics.

In our research we aim to improve cancer immunotherapies potential by understanding:

1) the complex regulatory network in immunological synapse that modulates the response;

2) how gene expression changes driven by genetic variability translate to alterations in immunological synapse regulatory network.

We developed a computational method that infers gene interaction networks from expression profiles in large cohorts of patients using unsupervised Bayesian machine learning model. Our method also reduces the dimensionality of data producing factors, that represent differences between cancer sub-types and individual patients.

In this study we compare regulatory networks in subtypes of kidney carcinomas: clear cell adenocarcinoma, papillary adenocarcinoma, and renal cell carcinoma. We found significant differences between subtypes, with some key nodes of network being not expressed, while others being significantly up-regulated. These type-specific alterations of immunological synapse gene network may explain difficulties in developing successful checkpoint inhibitors immunotherapy in kidney carcinomas. It also indicates that different subtypes may require targeting different checkpoint proteins to facilitate an effective therapy.

P. Stempor: None. **P. Dobosz:** None.

P16.12D

Deciphering complex genomic rearrangements by traveling through 3rd generation sequencing in rare diseases: two chromothripsis cases

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Background: Genome sequencing (GS) is rapidly becoming the standard approach to identify the molecular cause of rare Mendelian genetic disorders, allowing the simultaneous analysis of single nucleotide and structural genomic variants

(SV). However, technical limitations are associated with the use of short reads (srGS) in the detection of SV, especially in case of breaking points involving repetitive elements. Hence, long read sequencing (lrGS) represents a promising tool expected to overcome this problem.

Method: We deployed 30X srGS and 20X SMRT sequencing (Pacific Biosciences) to evaluate the utility of lrGS to resolve complex SV in 2 patients with syndromic intellectual disability and carrying respectively more than 30 and 70 chromosomal rearrangements (chromotripsis).

Results: Using 10 *in silico* generated datasets containing known SV, were tested a combination of 6 mapping tools (blasr, bwa, nglmr, Lorfast, pbmm2, minimap2) and 3 SV callers (PBhoney, sniffles, pbsv) to identify the outperforming pipeline. We then applied our pipeline to the 2 chromothripsis and, using SV length and population frequency information available from publicly data, we filtered out common variants. Retained breakpoints were independently validated by PCR, Sanger sequencing and/or FISH. Next, we tested 3 assembling tools (Falcon, Canu and wtdbg2) to perform *de novo* derivative chromosome reconstruction. Using outperforming parameters, we obtained 10238 contigs with a N50 at 13 Kb and a maximum contig length of 11Mb.

Conclusions: Overall the combination of srGS and lrGS permitted to resolve, complex chromosomal breakpoints and to reconstruct the phasing of structural variations, not otherwise achievable by srGS alone.

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P16.13A
Gene-set burden analysis detects novel signals in whole exome sequencing of a chronic kidney disease cohort

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Exome and genome sequencing of large disease cohorts allows researchers to investigate the role of rare variants in complex, heterogeneous disorders, providing new understanding of disease and potentially identifying novel drug targets. Using gene-based collapsing analysis of individuals sequenced through the AstraZeneca Medimmune Genomics Initiative, we previously validated known disease genes and identified new suggestive signals in specific genes. However, risk to complex disorders may also be driven by multiple hits in a common pathway, or the scenario where many genes can cause the same disease thereby making it difficult to significantly detect individual genes in currently available cohort sizes.

We present here the application of "mega-gene" as a new analytical approach to identify biologically relevant gene-sets involved in disease. We achieve this by comparing the case and control rates of genes in a given gene-set that carry rare and deleterious-predicted variants. The tested gene-sets include 10,161 from published databases such as KEGG, Reactome, Gene Ontology and others. Applying the novel approach to a cohort of individuals who were diagnosed with chronic kidney disease and recruited into an AstraZeneca clinical trial, we find several gene-sets achieving a false discovery rate (FDR) adjusted p-value < 0.01. Further investigation is required to determine if and how the gene-sets, and the underlying individual genes, might be relevant to disease. This demonstrates an example of an ontology-, network- and pathway-centric approach to explore the genetic architecture of heterogeneous genetic disorders.

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P16.14B
mantis-ml: Disease agnostic gene prioritisation with automated machine learning

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Faced with the petabytes of genomic data generated through our Genomics Initiative towards our ambition of analysing up to two million genomes, we have implemented novel multi-dimensional, multi-step machine learning frameworks to holistically and objectively assess the relevance of genes across multiple therapeutic areas. Instead of laboriously triaging highly-ranked possible targets from high-throughput genome-wide screens, we can now draw on Artificial Intelligence to cut the inevitable subjectivity out of conclusions from manual literature review of multiple data sources. We sought to address this by exploring a plethora of gene-associated annotations (including HPO, ExAC, GTEx, model-organism phenotypes and genic-

intolerance scores) to identify hidden patterns among disease-associated genes and predict novel genes of interest. We developed *mantis-ml*, an automated machine learning (AutoML) framework, which employs Extremely Randomised Trees and Positive-Unlabelled learning to rank known and novel disease-associated genes through iterative training and prediction sessions on random balanced datasets across the entire gene set (n=18,225). We applied this framework on two major disease categories: cardiovascular and chronic kidney disease. *mantis-ml* successfully ranked well-established disease-associated genes in the top 0.01-0.1% of all genes associated with each disease, respectively, and achieved an average AUC performance of 0.82-0.83. Notably, the most highly ranked novel genes were found to be enriched for characteristics relevant to the respective disease, based on expression and pathway analysis using the 'Ingenuity Pathway Analysis' tool. Coupled with high-throughput genomic screens, we believe that *mantis-ml* will enhance our understanding of complex disease genotype-phenotype associations, accelerating the path to successful target identification and validation.

D. Vitsios: None. **S. Petrovski:** None.

P16.15C
Defining the landscape of circular RNAs in multiple sclerosis

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Introduction: Circular RNAs (circRNAs) are a new class of non-coding RNAs that are emerging as important players in the pathogenesis and progression of autoimmune and neurological diseases, such as multiple sclerosis (MS). We previously demonstrated an enrichment of circRNAs at MS genome-wide associated loci as well as a genotype-dependent regulation of a circRNA derived from the MS-associated *STAT3* gene. Here, we aimed at exploring the global profile of circRNA expression in MS patients and evaluating a possible correlation with their genetic background.

Materials and Methods: We performed RNA-seq experiments on RNAse R-treated RNAs, extracted from peripheral blood mononuclear cells of 10 MS patients and 10 matched healthy controls. The same patients/controls were genotyped using the Infinium HumanCore-24 v1.1 BeadChip. An expression quantitative-trait loci (eQTL) analysis, aimed at correlating the genotypes of MS-associated variants with the expression levels of circRNAs, was performed using the FastQTL tool.

Results: We detected 5,663 circRNAs, uniformly distributed on all chromosomes. The expression analysis revealed 166 differentially expressed circRNAs in MS patients ($P<0.05$), 125 of which are downregulated. Notably, the dysregulation of one of the top hits has already been confirmed in an independent case-control cohort. Moreover, the eQTL analysis evidenced a significant association between 58 MS loci and the expression of at least one circRNA.

Conclusions: This work established for the first time a complete profile of circRNA expression in MS, suggesting that MS-associated variants may influence the expression levels of circRNAs, acting as "circ-eQTLs".

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P16.16D
A multi-site comparison of copy number variant callers for germline next generation sequencing using targeted capture

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Introduction: Using targeted Next Generation Sequencing (NGS) to detect Copy Number Variants (CNVs) would allow for increased throughput and decreased costs in clinical genetics; however, this is challenging with small CNVs (affecting one or few exons). To date, a wide comparison of CNV-callers has been limited by the paucity of samples with known small CNVs. Here we carry out a comprehensive comparison of CNV callers.

Materials and Methods: CNV-caller scripts and docker containers were created to carry out a consistent analysis of data. These have been shared with 5 NHS genetics laboratories, using local NGS-data with a known CNV-status. The final data will be pooled, with 200 CNV-positive samples expected. Preliminary analysis was done using the ICR96 exon validation series dataset (with known CNV-status).

Results: In the preliminary results (Table 1) DECoN performed best, though there is little difference in the 95% confidence intervals between DECoN, ExomeDepth and

GATK. Canvas and WISExome were also tested but were not applicable for targeted NGS.

Table 1: Preliminary results using the ICR96 Exon Validation Series data

Caller (version)	Number of CNVs detected	Sensitivity (95% confidence interval)
CNVkit (0.9.5)	11	0.20 (0.11 - 0.34)
CopywriteR (2.14.1)	31	0.57 (0.43 - 0.70)
DECoN (1.0.2)	52	096 (0.86 - 0.99)
EXCAVATOR2 (1.1.2)	0	0 (0 - 0.08)
ExomeDepth (1.1.10)	51	0.94 (0.84 - 0.99)
GATK gCNV (4.1.0.0)	51	0.94 (0.84 - 0.99)
XHMM (1.0)	4	0.07 (0.02 - 0.19)

Conclusions: A comprehensive analysis is underway and will give a high-confidence comparison of CNV-callers. These results will identify the most suitable CNV-caller in clinical genetics.

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P16.17A
SnakeLines: integrated set of computational pipelines for paired-end sequencing reads

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Introduction: Development of sequencing technologies with massive parallel throughput allowed laboratories around the world to analyze DNA fragments of various organisms. However, actual use of DNA sequencing data is impeded by two main obstructs. Firstly, DNA data processing and interpretation is often difficult for researchers and clinicians with small computational background. Secondly, due to the complexity of an available bioinformatic software, where one tool is dependent on specific version of another tool, reproducing data analyses in other computational clusters is often too troublesome.

Results: We propose a framework together with an implemented set of computational pipelines, called SnakeLines, for processing of paired-end Illumina reads, including mapping, assembly, variant calling, viral identification, transcriptomics, metagenomics, and methylation analysis. Our framework implements a self-created virtual environment that contains required tools and libraries and isolates them from host operating system, thus ensuring easy portability and reproducibility across different Unix-based systems.

Availability: The open-source code of the pipelines, together with test data, is freely available for non-commercial use from https://github.com/jbudis/snakelines.

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P16.18B
Intraspecific tolerance of nonsynonymous variation is closely correlated between human and mouse orthologues

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Genic constraint describes how tolerant a gene is to nonsynonymous variation before it is removed from the population by negative selection. The recent abundance of sequence data has enabled the development of multiple metrics to evaluate gene constraint in human populations, and such efforts have discovered an enrichment for disease-associated genes amongst those genes that are most

constrained. Notably, genic constraint has not been estimated for mouse, which is the most widely utilised mammalian model organism for biomedical research. As a result, the relationship between gene constraint in human and mouse orthologues remains poorly understood. Here, we calculate constraint scores for mouse genes and show constraint is positively correlated between human and mouse orthologues (r = 0.806). We further assess the relationships between mouse gene constraint and knockout phenotypes, showing gene constraint is positively correlated with an increased number of phenotypic annotations, in addition to an enrichment in developmental, morphological, and neurological knockout phenotypes amongst the most constrained genes. Finally, we show mouse constraint can be used to predict human genes associated with Mendelian disease, and is positively correlated with an increase in the number of known pathogenic variants in the human orthologue. Our research supports the role of mouse models for understanding the mechanistic basis of gene function and human monogenic disease.

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P16.20D
Efficient pipeline for CNV detection from targeted NGS data

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Introduction: Bioinformatic tool development for analysis of copy number variants (CNV) from NGS data lags behind that for other variant types. We present here an efficient bioinformatic pipeline for CNV detection from targeted NGS data. This will increase the diagnostic yield in a cost-effective way in clinically and genetically heterogeneous disorders, such as neuromuscular disorders.

Materials and Methods: We chose four programs, CoNIFER, XHMM, ExomeDepth and CODEX, since they have complementary CNV detection ranges in sensitivity, specificity, and variant size and state. Heterozygous deletions and duplications were generated *in-silico* into samples sequenced with a gene panel of approximately 350 genes for neuromuscular disorders. A logarithmic regression model was trained for predicting true positive CNVs among variant detections from these samples. This model was validated with negative (N=30) and positive (N=52) control samples with real CNVs.

Results: CNV features, such as state or size, did not markedly affect the model performance. Therefore, the simplified model examines only program scores for detections. Model with detections from all four programs provided more accurate CNV detection results for the control samples than any other program combination or program alone.

Conclusions: No single analysis program can capture all CNV sizes and types with equal accuracy. Therefore, a combination of programs should be used to maximize sensitivity and specificity. Additionally, the variants should be reviewed with a statistical model to streamline and standardize the process of filtering of variants for variant annotation and clinical use.

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P16.21A
DECIPHER: Enabling the sharing and interpretation of rare disease variants and associated clinical phenotypes

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DECIPHER (https://decipher.sanger.ac.uk) established in 2004 has grown to become a major global platform for the visualization of phenotypic and genomic relationships and for sharing linked-anonymised rare disease patient records. DECIPHER displays both the nuclear and mitochondrial genomes, and all scales of genomic variation, from single base to megabases, in a single interface. DECIPHER's mission is to map the clinically relevant elements of the genome and understand their contribution to human development and disease.

DECIPHER stores both phenotype and genotype data. Currently there are more than 37,000 variants (sequence and copy-number) and more than 89,000 phenotypes (HPO terms) that are shared openly on the website. The wealth of these data in DECIPHER allows the aggregation of data

associated with pathogenic variants in disease genes. To aid diagnosis aggregated phenotype data is used to identify the most discriminating phenotypes associated with disease genes. Quantitative phenotype data, such as anthropometric measurements and developmental milestones, are aggregated and visual/statistical tools are used to allow the comparison of a patient with other patients harboring pathogenic variants in the same gene. Aggregating data in DECIPHER is extremely powerful in aiding diagnosis and discovery whilst harmonized with DECIPHER's proportionate data sharing approach.

DECIPHER is dedicated to the sharing of patient data for discovery and diagnosis and is a pioneering partner in the Global Alliance for Global Health (GA4GH) and founder member of the Matchmaker Exchange (MME) which enables the federated discovery of similar entries in connected databases.

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P16.23C
Evaluation of the Bionano optical mapping technology as a replacement of conventional cytogenetics in a diagnostic setting

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The diagnostic remit of a clinical cytogenomic laboratory is to identify DNA copy number variations (CNVs) and structural variations (SVs) that are likely to impact phenotype.

Current methodologies have significant limitations. Within the South East Scotland Genetic Service, chromosomal microarray (Affymetrix 750k) is used to diagnose CNVs greater than 10 kilobase (Kb) in size, but cannot detect "balanced" aberrations such as translocations or inversions. G-banded chromosome analysis detects most structural variation, but at a lower resolution of 3-5 Megabases (Mb) and Fluorescent In Situ Hybridisation (FISH) often requires prior knowledge of the variant.

Sequencing technology has improved, with significant progress in the detection of single nucleotide changes and short indels, but most methods produce short-read sequences and therefore fail in GC rich or repetitive regions and cannot detect larger variants accurately.

Here we present the results of a small validation series using Bionano's Saphyr system, an optical mapping technology that utilises single, megabase-size native DNA molecules, identifying a custom recognition sequence to use fluorescent labelling for alignment. The automated calling algorithms aim to detect all copy number and major structural variation types (including balanced rearrangements), potentially replacing the need for multiple analytical techniques.

A range of sample with known abnormalities (analysed in house using a combination of microarray, G-banding and FISH) were analysed using optical mapping to assess the clinical utility of the system. The Bionano optical mapping system not only detected all copy number variants with a higher resolution than microarray, it also detected all balanced structural variants.

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P16.24D
Conserved clinical variation visualization tool (ConVarT)

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Introduction: It is extremely challenging to visualize the evolutionary conservation of amino acid substitutions associated with diseases because there is no available tool that maps the coordinates of these amino acids of humans in common model organisms. Recent technological advances in DNA sequencing have led to a comprehensive list of human genetic variations, the functional effects of many of which are yet to be clarified. Model organisms could be employed to functionally assess the effect of genetic variations. Here, we designed a user-friendly tool that allows to visualize the comparability of human genetic variations across the species and currently we are testing the functional impact of disease associated amino acids with CRISPR in *C. elegans*.

Materials and Methods: We extracted the genes consisting of all types of amino acid substitutions from ClinVar database. We have performed multiple sequence alignment across most commonly used model organisms and integrated amino acid substitutions into corresponding positions. We are currently integrating the recently published gnomAD databases into our database.

Results: ClinVar data has revealed that while over 50000 genetic variations are pathogenic, large portions of human genome variations (at least 40%) on genes are annotated with uncertain clinical significance, suggesting that the functional assessment of these positions are needed. We are currently performing statistical analysis to find the comparability of human genetic variations among other species.

Conclusion: Our tool can serve to visualize the evolutionary conservation of disease causing amino acids

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P16.25A
Open Targets Genetics: An open-science resource for improved drug target identification and prioritisation using genetic data

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The discovery and development of new pharmaceuticals is a time consuming and expensive process. A major challenge is high attrition, as a large proportion of drugs are found to be ineffective at clinical trial. However, drugs that have genome-wide association study (GWAS) evidence supporting a link between its target and indication are twice as likely to be approved for clinical use. Here we present Open Targets Genetics, an open-science resource which makes robust connections between disease-associated loci and likely causal genes, to enable the identification and prioritisation of new drug targets.

We combine GWAS associations curated from literature and the recently released NHGRI-EBI GWAS Catalog summary statistics database (including over 3,000 UK Biobank phenotypes) to locate over 100,000 disease-associated loci. We then use statistical fine-mapping (or linkage-disequilibrium expansion if no summary statistics are available) to identify a set of potentially causal variants at each locus. Lastly, variants are integrated with functional genomics data (e.g. promoter capture Hi-C, DNase hypersensitivity sites), molecular quantitative trait loci (e.g. eQTLs, pQTLs) and *in silico* functional predictions to link each variant to its target gene(s) using a unified evidence score. Most recently, we have added systematic disease-molecular trait colocalisation analysis using the upcoming Open Targets-European Bioinformatics Institute eQTL database.

The results of Open Targets Genetics are made available through our web portal (genetics.opentargets.org), for bulk download and via a GraphQL API, enabling users to easily prioritise genes at disease-associated loci and assess their potential as pharmaceutical targets.

E. Mountjoy: None.

P16.26B
Complete workflow for Discovery and Verification of eQTLs in Lung Adenocarcinoma

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Genome wide association studies (GWAS) have revealed associations between genotype, phenotype and pathologies. Expression quantitative trait loci (eQTLs) have been mapped in many tumor types, such as breast, lung and prostate cancer. These studies measured genome-wide gene expression in tumors and identified associations between these gene expression levels and commonly inherited genetic variants profiled in the same patients. Since the majority of inherited cancer risk variants as identified by GWAS are in non-coding regions, eQTL analysis of cancer tissues is becoming extremely important. In this study we demonstrate a streamlined workflow to study eQTLs in lung adenocarcinoma. Previous GWAS have identified several lung cancer susceptibility loci, however functionality of most of these loci remains unexplained. Here we focused on SNPs previously reported to be associated with lung cancer in different populations. We studied the association of candidate SNPs with lung adenocarcinoma risk and overall survival in a cohort of 96 patients and healthy controls using the Axiom genotyping arrays and Clariom D gene expression arrays. The function of these SNPs was assessed as eQTLs, these results suggest that the candidate SNPs exert their effects on cancer risk/outcome through the modulation of mRNA levels of their target genes.

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P16.27C
Expanding GENCODE gene annotation to elucidate disease-linked variants

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An investigation into the link between variant and phenotype usually begins with gene annotation. However, most GWAS variants fall in non-transcribed regions, and even exon-focused catalogs such as ClinVar contain thousands of variants that do not overlap known transcripts. Here, we present efforts by GENCODE to improve the scope and usability of our human gene annotation in a disease-related context, along multiple lines. Firstly, our public geneset is about to undergo a massive expansion, as tens of thousands of new transcript models are incorporated based on long-read sequencing datasets. This work will cause thousands of variants to be reclassified as transcribed. However, even when variant transcription is established, uncertainties regarding 'functional categorization' - especially coding potential - can still undermine attempts to prioritize variants or judge pathogenicity. This expansion is therefore being carried out alongside efforts to reappraise the CDS content of our geneset, incorporating evolutionary conservation, population genetics datasets and proteogenomics methods. Pertinently, our creation of the first whole-genome PhyloCSF dataset allowed us to add over 230kb of novel high-quality CDS annotation, leading to the reclassification of 118 GWAS variants from non-coding to protein-disrupting. Furthermore, we are now utilizing a fully integrated manual workflow to develop our novel annotations in a clinical context. For example, our targeted reannotation of 191 genes linked to epilepsy led to the identification of 3 novel *de novo SCN1A* coding mutations within a panel of 122 patients with Dravet's syndrome.

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P16.28D
The Mouse Genome Informatics (MGI) knowledgebase: new features enhancing anatomical comparison of gene expression and phenotype profiles

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Mouse Genome Informatics (MGI) is the key knowledgebase for the laboratory mouse. MGI integrates mouse genotype-phenotype datasets from biomedical literature and large-scale projects with genomic, mutation, expression, functional and human disease model data to inform disease etiology and therapeutic design. Recent enhancements include newly established anatomical mappings between the Mammalian Phenotype (MP) and Mouse Developmental Anatomy (EMAPA) ontologies and a user interface, co-deployed with the Gene Expression Database (GXD), which enables comparison of gene expression and phenotype annotations for mouse genes. The new Gene Expression + Phenotype Comparison Matrix, accessible from MGI's Gene Detail page, visually juxtaposes tissues where a gene is normally expressed against tissues where mutations in that gene cause phenotype abnormalities. The anatomy axis of the matrix can be expanded and collapsed, allowing users to interactively explore correlations between gene expression and phenotype data at different levels of spatial resolution. An enhanced Mouse Developmental Anatomy Browser provides quick access to phenotype annotations in addition to expression data associated with a given anatomical structure or substructures. The updated MP Browser links to mapped anatomical structures, enabling access to wild-type expression data in tissues associated with a given phenotype. Moreover, MGI's Allele Detail page features a link to affected anatomical structures, facilitating retrieval of wild-type expression data in tissues afflicted by the underlying mutation. The MGI features presented here will enhance researchers' ability to probe gene function and gain critical mechanistic insights into developmental and disease processes. Supported by NHGRI grant HG000330 and NICHD grant HD062499.

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P16.29A
Deconvoluting the Dementia phenotype using functional computational approaches

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Introduction: The development of Alzheimer's disease (AD) is a multi-causal process, with an interaction between components. In this respect, delineating the constituent biological components that play part will bring a greater

understanding of the processes in action. We will present an integrative computational approach of linking gene-expression to phenotypes and biological pathways in *Drosophila* and extend the methodology in individuals with AD. We base our approach on the concept that a broad phenotype, such as AD, is composed of simpler intermediate subphenotypes.

Methods: By combining gene-expression data from single-gene mutants in *Drosophila* that exhibit a particular subphenotype (biological replicates of this phenotype), genes/biological pathways that play a role in the subphenotype will be enriched. The goal is to enhance the signal from genes that play a role in a subphenotype and reduce the noise. Linear mixed-effect model will be used to circumvent the inter-experiment variability and to combine the gene-expression data and generate molecular signatures from different experiments and accounting for differences that are not of interest.

Results: We will present preliminary, proof-of-concept results for several complex subphenotypes in *Drosophila*. We will also show how these molecular signatures can be used to describe the studied subphenotypes.

Conclusions: Our methodology has the ability to not only assert the relationship of AD with already known subphenotypes, but can also discover not yet tested subphenotypes and associated biological pathways. This methodology has also a potential to quantify the relationship between different subphenotypes and AD animal models.

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P16.31C
F508del correction in CFTE29o- cell line by CRISPR/Cas9

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Introduction: In most cases cystic fibrosis (CF) is incurable disease, which makes the development of gene therapy an actual task. Genome editing provides wide opportunities for modeling and treating CF. The aim of this work was editing of F508del mutation in CFTE29o- cells using CRISPR/Cas9.

Materials and Methods: The work was performed in CFTE29o- cell - immortalized cell line of tracheal epithelium from a patient with CF (F508del/F508del). Three nucleases (eSpCas9(1.1), SpCas9(HF4), SaCas9) in combination with four sgRNAs were used. Two sgRNAs were targeted specifically to F508del (sgCFTR#1, sa_sgCFTR#3); the other two were to downstream region (sgCFTR#2, sgCFTR#3). In addition, we used a plasmid with an insert of *CFTR* fragment with F508del to study potential influence of the genomic context on the editing efficiency. Four single-stranded oligodeoxynucleotides (ssODNs) were designed to repair double-strand DNA breaks. Cas9+sgRNA plasmids were co-transfected with model plasmid and ssODN into CFTE29o- cells by lipofection. The editing efficacy was evaluated by TIDE and TIDER methods.

Results and Conclusions: Indel formation efficacy was 2.1-7.5% in the plasmid (the highest efficacy with Cas9 (HF4)/sgCFTR#1) and 1.4-7.9% in the genomic locus (the highest efficacy with Cas9(1.1)/sgCFTR#3). Then we co-transfected cells with the most effective combination Cas9 (1.1)/sgCFTR#3 together with ssODN, homologous repair (CTT insertion) in genomic locus was 8.7%. This level of efficiency makes reasonable development of treatment of CF by genome editing. Additional studies are necessary to confirm these results and increase efficacy. This work was supported by Russian Science Foundation (Agreement No. 17-75-20095).

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P16.32D
Prospective interest in deploying multi-omics approaches to solve unsolved patients with suspected monogenic developmental delay syndromes

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Background: Exome sequencing (ES) represents the first-tier diagnostic test in patients presenting with syndromic developmental delay with suspected monogenic etiology. Yet, 50-70% of these patients remain unexplained at the molecular level, arguing the interest to extend the genetic investigations beyond protein-coding regions and to integrate multi-omics approaches.

Method: We launched a multi-centric study gathering 30 unsolved patients, after trio ES and array-CGH analyses, presenting with heterogeneous mild to severe syndromic intellectual disabilities. We performed trio genome sequencing (GS) combined with linked-read sequencing (10X Genomics) and blood-derived transcriptome analysis.

Results: We present our results on the first third of the cohort. GS analysis identified 3 *de novo* pathogenic or likely-pathogenic variants in *PURA, FOXG1, CYFIP2* and *KMT2D*, as well as an inherited hemizygous *FGD1* variant. The first two variants had not been captured by the exome kit, while *CYFIP2* was not yet a morbid gene at the time of the ES analysis. The remaining ones, had not been identified by the molecular diagnostic laboratory responsible for the ES analysis. GS highlighted the presence of a *de novo* 2 Mb inversion located 400 Kb upstream *MEF2C* (confirmed by linked-read analysis), responsible for the disruption of the Topologically Associating Domain regulating this gene, and a 9 Kb *de novo* complex structural variant, causing the heterozygous loss of 3 exons of *CASK* resulting in a frameshift mutation (RNA-seq data).

Conslusions: Our preliminary results on 10 patients show that a multi-omics approach allowed the identification of 4 additional molecular diagnoses not otherwise achievable with ES.

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P16.34B
Matched annotation from the NCBI and EMBL-EBI (MANE): Converging on a high-value, genome-wide transcript set

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Accurate annotation of the human genome is essential for genomics research and clinical applications. RefSeq (NCBI) and Ensembl/GENCODE (led by EMBL-EBI) produce independent human gene annotation. Since 2005, the joint Consensus Coding Sequence (CCDS) project has defined over 30,000 CDS for 95% of coding genes. However, the large number of alternatively spliced transcripts and the lack of standardized default transcripts displayed across resources present challenges, especially in the clinical context. Building on our past collaboration, we have

launched a new initiative, the Matched Annotation from NCBI and EMBL-EBI (MANE) project, to jointly converge on a high-confidence, genome-wide transcript set.

During phase-1, we will release the MANE Select transcript set to include one well-supported transcript per protein-coding locus. All transcripts in the set will perfectly align to the GRCh38 reference assembly and represent 100% identity (5'UTR, CDS, 3'UTR) between the Ensembl (ENST) transcript and the corresponding RefSeq (NM) transcript. MANE Select transcripts are identified using independent computational methods complemented by manual review and discussion. The methods utilize evidence of functional potential such as expression levels, evolutionary conservation, and clinical significance. Transcript ends are defined using CAGE data from the FANTOM consortium and polyA site data from conventional and next generation sequencing. Currently, we have defined MANE Select transcripts for 53% of human coding genes and are working towards genome-wide coverage. During phase-2, we intend to release an expanded set (MANE Plus) to include additional transcripts per locus that are well-supported or of particular user interest.

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P16.35C
Full p-value summary statistics in the GWAS Catalog

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The NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog, www.ebi.ac.uk/gwas) contains over 5,500 full p-value summary statistics (SS) datasets, making it the largest repository of GWAS SS. To ensure SS are consistent across studies and interoperable they are pre-processed and made available in a standard format. They are also harmonised, with respect to genome build and strand. Each SS dataset is linked to the curated data and metadata from the publication. Older GWAS Catalog studies are re-curated, where required, to ensure the study structure is correctly represented.

Access to SS is provided from the GWAS Catalog FTP site (www.ebi.ac.uk/gwas/downloads/summary-statistics), via API from a SS datastore (www.ebi.ac.uk/gwas/summary-statistics/docs/) and via links within our redesigned query interface (www.ebi.ac.uk/gwas/search). The query interface also provides improved search and display functionality for all Catalog data. New pages provide access to structured information and visualisations that were previously unavailable, while a new API provides programmatic access to curated Catalog data (www.ebi.ac.uk/gwas/docs/api).

We are working to increase the number of available SS, encouraging authors to submit their SS and campaigning for data sharing. To ensure SS are accessible, usable, updated and relevant to the user community we are engaging with the community to develop standards for data reporting. The broad availability of SS will vastly extend the potential of GWAS, allowing users to perform a wide range of analyses.

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P16.36D
Genetic control of fatty acids in the isolated population of Sardinia

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It is well established that short and long chain fatty acids are the main medium of interaction between immune system and gut microbiota. Although there are preliminary results on the genetic and environmental influence on the metabolite levels, the interactions between immune systems

and microbiome are very complex and their mechanisms still need to be understood.Here we present an omic analysis of the fatty acid levels in the isolated population of the Sardinia island. The cohort involves ~8,000 individuals from the valley of Lanusei, deeply genotyped with >23M genomic variants imputed from a population-specific reference panel. In addition to >1,000 quantitative traits biomedical parameters, the cohort is also deeply phenotyped with >2,000 immune cell traits. We performed a GWAS analysis on levels of 8 short and 29 long chain fatty acids in 2,000 volunteers of the SardiNIA cohort (ranging from 19 to 103 years of age). The association results showed signals in the *ACADS, SLC44A5* and *ELOVL2* gene regions. Furthermore, we observed a general increase of fatty acid levels with age, with specific exceptions. Additional analyses combining the immune profile by means of correlations, colocalization, and mendelian randomization will provide hints on the regulatory functions of these important molecules. The results will help to elucidate the host genetic regulation on fatty acids variance and how and to what extent they interact with the host immune system in health and disease state.

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P16.37A
HiVA: a web platform for haplotyping and copy number analysis of single-cell genomes

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Haplotyping is imperative for comprehensive analysis of genomes, imputation of genetic variants and interpretation of error-prone single-cell genomic data. We have developed a pipeline and user interface for single-cell analysis named HiVA (https://hiva.esat.kuleuven.be). HiVA (Haplarithm inference of Variant Alleles) is an interactive web platform for genome haplarithmisis of DNA samples derived from a large number of cells down to a single cell. HiVA automatically reconstructs parental haplarithms (i.e. haplarithm profiles indicating both haplotypes and copy number states) and provides a user-friendly interface for scrutinizing allelic imbalances across the genome. We are showing that HiVA enables concurrent haplotyping and copy-number profiling of single cells. In contrast to conventional family-based haplotyping methods that make use of discrete bi-allelic SNP genotypes (AA, AB and BB) to reconstruct haplotypes, haplarithmisis uses continuous SNP genotypes values, which potentially harbor quantitative (haplotype) and qualitative (copy number) assessment of genomes. HiVA is a novel sequencing-based approach for whole-genome SNP typing of single cells, and determine genome-wide haplotypes, the copy number of those haplotypes as well as the parental and segregational origin of chromosomal aberrations from sequencing- and array-based SNP landscapes of single cells.

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P16.38B
HADA: a resource for automated annotation of next-generation sequencing data in hereditary angioedema studies

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Introduction: Hereditary angioedema (HAE) is a rare genetic disease caused by dysfunction of the C1 inhibitor or dysregulation of the kinin cascade. Because of its non-specific signs, HAE is poorly recognized in the clinical field, resulting in delayed diagnoses that increase the risk of morbidity and mortality. To adapt its diagnosis to next-generation sequencing (NGS)-based assessments, we developed Hereditary Angioedema Database Annotation (HADA) tool.

Materials and Methods: We performed a manual curation and update to GRCh37/hg19 of the causal variants

described to date in HAEdb and VarSome and classified all of them to the ACMG guidelines. The information was used to establish a database configured for ANNOVAR to be seamlessly incorporated into the routine NGS bioinformatics annotation.

Results: Currently, HADA includes records for 416 simple causal variants in the five genes underlying HAE. However, 25% of those were classified as non-pathogenic by current guidelines, suggestive of a gap in the interpretation of disease variants and a necessity to improve the classifiers of pathogenicity potential of genetic variants.

Conclusions: To adapt the genetic diagnosis of HAE to the era of NGS-based genomic medicine, we have developed HADA as a freely available tool simplifying the identification of point mutations and short indels causing HAE. Annotation of structural variation will be covered in future developments. Funding: Ministerio de Ciencia, Innovación y Universidades (RTC-2017-6471-1; MINECO/AEI/FEDER, UE), agreement OA17/008 with ITER to strengthen scientific and technological education, training, research, development and innovation in Genomics, Personalized Medicine and Biotechnology, and a CajaSiete-ULL fellowship (to AMA).

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P16.39C
HLA-VBSeq v2: enhancements of HLA calling accuracy from WGS data with full-length Japanese HLA sequences

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Introduction: The human leukocyte antigen (HLA) system, which is the most variable gene region in the entire genome, encodes the major histocompatibility complex (MHC) proteins in humans on chromosome 6 and has been reported to be associated with numerous immune-mediated diseases. HLA calling from whole genome sequencing data (WGS) remains to be challenging due to the incompleteness of population specific references. We have previously described the construction of Japanese reference sequences that fully covers the gene body region of HLA class I (Japanese HLA sequences). This study aims to evaluate the accuracy of HLA calling from WGS when the Japanese HLA sequences were combined with the IPD-IMGT reference sequences to estimate the most likely HLA genotypes.

Materials and Methods: To assess the usefulness of population-specific HLA references in HLA calling, we have compared the performance of different sets of references (with and without Japanese HLA sequences) across HLA-A, HLA-B and HLA-C alleles by using HLA-VBSeq, a flexible software which allows the use of arbitrary reference sequences.

Results: We evaluated their performances by using 418 Japanese samples as testing data, in which HLA genotypes were determined with Luminex technology. All of the tests were implemented based on 4-digit resolution. As a result, the performance of HLA-calling in the Japanese population significantly increased with custom HLA panel

Conclusions: We suggest that including the sequences obtained from regional population samples is an effective way to improve the accuracy of HLA calling from WGS dataset. The software is available from (http://nagasakilab.csml.org/ja/hla-vbseq) as HLA-VBSeq v2.

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P16.40D
Efficient and high-throughput genotyping of elusive human polymorphic inversions mediated by inverted repeats

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Structural variants are large genomic rearrangements that affect a big number of bases, with the potential to have a wide phenotypic impact. Among them, inversions are genomic regions that change orientation without gain or loss of DNA and many of them have highly identical inverted repeats (IRs) at the breakpoints. Moreover, they tend to be recurrent and not linked to SNPs. Thus, the complexity of such regions has made inversion characterization difficult. Previously, we developed a combination of inverse PCR and MLPA to simultaneously interrogate 24 common human inversions mediated by IRs in hundreds of individuals. Here, we have optimized this method by taking advantage of the iPLEX technology to detect one single nucleotide change specific of each orientation using mass spectrometry and extending the analysis to additional

inversions. Also, we have improved genotype accuracy by designing specific assays that take into account common polymorphisms affecting restriction-enzyme target sites. Currently, we are able to genotype 37 inversions amplified in four different PCR reactions from as little as 400 ng of DNA. In order to test the performance of the method, we have genotyped 95 individuals from three populations of the 1000 Genomes Project with 94.3% genotyping rate and an error rate of ~3% for a single experiment. The new genotype data generated allow us to have a first glimpse on the functional and evolutionary consequences of these inversions. In addition, having a high-throughput genotyping technique paves the way to unravel the role of inversions in complex traits and disease susceptibilities.

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P16.41A
Evaluating the expansion of the experimentally determined human protein interactome using the PICKLE meta-database

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Introduction: The Protein InteraCtion KnowLedgebasE (PICKLE; www.pickle.gr) is a meta-database of the human experimentally-determined direct protein-protein interaction (PPI) network, integrating five primary datasets over the genetic information ontology network of the UniProtKB/Swiss-Prot reviewed human complete proteome (RHCP). Its ontological PPI integration using RHCP as a standardized reference node set constitutes PICKLE the only PPI meta-database enabling the evaluation of the human interactome expansion at both the protein and gene levels through comparison of its successive releases; this evaluation is the objective of this study.

Methods: The default ("cross-checked") Uniprot-level interactomes of PICKLE 2.1-2.3, integrating data from 02/2015 to 11/2018, respectively, were compared. Default networks exclude PPIs of low experimental confidence of being direct. Network analysis was performed using Cytoscape.

Results: The current human experimentally-determined direct PPI network, as reconstructed by PICKLE, comprises 178306 PPIs between 15823 UniProt IDs, twice more than the largest primary datasets, emphasizing thus the need for source database integration for reliable human interactome reconstruction. The increase in newly added proteins is one-third than that in newly determined PPIs, most of the latter enriching the interactions of existing proteins, while, as predicted in PICKLE 1.0, most newly added proteins have 1-4 interactions. The interactome becomes denser, however, its expansion over the 15% of RHCP with no known PPIs may require targeted experiments.

Conclusions: The study supports our previous statement that the human protein interactome structure with respect to its hubs has been largely defined. Elucidating its dynamics is the next challenge for network medicine.

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P16.42B
Gene co-expression network rewiring between pre-symptomatic and Huntington's disease human blood samples

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Introduction: Huntington's disease (HD) is a rare, inherited autosomal dominant neurodegenerative disease. Network based bioinformatics approaches are used to highlight the genes that participate in the co-expression network differentiation between pre-symptomatic and HD status.

Materials and Methods: A microarray dataset "Human blood expression for Huntington's disease versus control" (GSE1751), was obtained from Gene Expression Omnibus. Differential expression and gene co-expression analysis took place with Limma, Parmigene and igraph packages. DyNet, a Cytoscape plugin, was used to visualize the most rewired nodes and edges in pairwise network comparisons, namely control vs HD and control vs pre-symptomatic HD gene co-expression networks. The highlighted genes through DyNet were further analysed through Pathway-Connector, a post-pathway analysis tool developed by our group, that provides clusters of connected pathways related to the input genes.

Results: From the top network-based differentiated genes we kept the ones that correspond to each network and we performed PathwayConnector analysis. Among the common pathways between HD and pre-symptomatic, we highlight the dopaminergic synapse pathway. Furthermore,

there are 12 pathways found exclusively in the pre-symptomatic group and 6 in the HD group.

Conclusion: The genes and pathways obtained through the presented network-based approach may be further investigated to understand which genes and proteins are most important in each stage providing insight regarding the corresponding implicated mechanisms.

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P16.43C
Analysis of genome sequencing data with a minimal investment IT-infrastructure

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The computational analysis of genomic data has become an integral part of genome research. The sheer volume of the data produced by whole-genome sequencing requires researchers to have access to sizeable IT-infrastructure in order to interpret the data produced. While large high-performance computing might be already available to larger research groups, especially smaller research groups would greatly benefit from a publicly accessible commercial alternative.

We demonstrate how to setup such an infrastructure with a small amount of time and a minimal initial investment using the Amazon Web Services (AWS) cloud infrastructure. After a walkthrough of the technical solutions used, the power of this setup is displayed by describing the analysis of a human WGS sample using the open-source analysis pipeline Sarek. Using Sarek on the AWS cloud infrastructure; mapping, germline variant calling and annotation for a human WGS sample (30X coverage) was performed for less than US-$ 50.

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P16.45A
Long read sequencing of patient-parent trios with intellectual disability

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Current short read whole genome sequencing (SRS) approaches are unable to identify all genetic variation in an individual. Long-read sequencing (LRS) techniques may resolve this problem by enabling more sensitive detection of structural variants (SVs) and variation in low-complexity regions. In order to confirm the potential increased sensitivity of LRS, we selected 5 patients with intellectual disability and their healthy parents and performed trio LRS using the Pacific Biosciences Sequel instrument. These samples had been previously analyzed extensively with CNV-microarrays, exome sequencing and by whole genome SRS, but no disease-causing variant was found. All samples were sequenced to at least 15x coverage, and a single trio was sequenced to more than 40x coverage. We found that more than 20Mb of the genome was covered only by LRS and not by SRS, of which 600kb within coding regions. Per individual genome we identified up to 22,000 SVs >50bp and >40,000 indels of 20-50bp. Mendelian inheritance concordance was as high as 87-95% within trios. Per trio we identified only 3-32 candidate de novo SVs. Additionally, we performed single nucleotide variant (SNV) calling, giving rise to 3-4 million SNVs per individual, with approximately 24,000 in the exome. Mendelian inheritance concordance for SNVs was as high as 97%. Taken together this shows that current LRS performs better for identifying SVs and variation in low-complexity regions, whereas results for SNVs are close to those for SRS. These results highlight the potential of LRS to replace SRS for clinical purposes in the future.

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P16.46B
LOY Associated Transcriptional Effect (LATE) in immune cells measured by single cell RNAseq and bulk RNAseq

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Mosaic loss of chromosome Y (LOY) in blood cells is the most common somatic human mutation and it is associated with all-cause mortality and increased risk for common disease such as cancer, Alzheimer's disease, CVD, AMD, autoimmune conditions and diabetes. Genes located on the Y chromosome, both in the male-specific region (MSY) and in the pseudoautosomal region (PAR), are involved in important processes such as transcription, translation, chromatin remodeling, ribosome assembly, transcription factor binding and post translational modifications. LOY therefore has the potential to cause genome wide dysregulation of gene expression in affected cells. Here we present results from transcriptome sequencing of freshly collected peripheral blood cells using two different technologies, i.e. scRNA-seq and bulk RNA-seq, the latter performed on selected immune cell fractions sorted using fluorescence-activated cell sorting (FACS). The results shows that: 1) LOY can be measured from RNA and that results are comparable with DNA-based measurements, 2) that the expression of genes located in the MSY and PAR regions of chromosome Y follows predictable patterns with lower expression as a consequence of LOY, and 3) that LOY Associated Transcriptional Effect (LATE) can also be identified in autosomal genes. The results indicate that LOY in immune cells cause dysregulation of gene expression with important functional consequences for affected cells and individuals. Together with previous findings that men with LOY in blood cells have a greater risk for mortality and disease, these new results helps explain why men live shorter lives compared to females.

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P16.47C
Mosaic loss of chromosome Y (LOY) dynamics over time, estimated with a new method

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The most common human somatic mutation is mosaic loss of chromosome Y (LOY), associated with many severe diseases and outcomes i.e. all-cause mortality, non-haematological cancers, Alzheimer's disease and diabetes amongst others. The method of choice for LOY estimation is SNP-array, because the generated data originally intended for GWAS is now used to for LOY studies. However one big drawback with SNP-array is the data-unit (log R Ratio), making it difficult to interpret and compare with LOY measurements using other methods.

Here I present a new method to transform SNP-array data into percentage of LOY cells. This method was derived from LOY measurements from 121 men at 93 years of age, using three different methods i.e. SNP-array, whole genome sequencing and droplet digital PCR targeting *AMELY* and *AMELX*. This standardization was applied to samples from a unique cohort consisting of LOY measurements from 276 men, sampled at different time points over a period of up to 22.2 years. Most of the individuals had an increase in measured LOY over time but complex LOY dynamics could also be seen, likely due to aberrant clonal expansions.

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P16.48D
Mendelian Randomization analysis of celiac GWAS reveals a blood expression signature with diagnostic potential in absence of gluten consumption

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Introduction: Celiac disease (CeD) is an immune-mediated enteropathy with a strong genetic component for which the main environmental trigger is dietary gluten. Being on a gluten-containing diet is necessary for a correct diagnosis. We hypothesize that merging different levels of genomic information through Mendelian Randomization (MR) can help discover genetic biomarkers useful in the diagnosis of different conditions such as CeD.

Methods: We apply a MR-based approach confronting results from the largest to-date genome-wide association study (GWAS) on CeD and from expression and methylation quantitative trait *loci* (QTL) databases. Particularly, we search for the overlapping hits of the different MR analyses performed and interrogate the prioritized genes in independent expression databases to test their diagnostic potential.

Results: We identify *UBE2L3*, an ubiquitin ligase located in a CeD-associated region whose expression is altered in peripheral mononuclear cells (PBMCs) of CeD patients on gluten-free diet (GFD). The relative expression of *UBE2L3* isoforms predicts CeD with 100% specificity and sensitivity and thus could be used as a diagnostic marker, especially in the absence of gluten consumption.

Conclusions: The clinical interest of this finding is in line with the rising frequency of both self-reported wheat sensitivity and auto-imposed GFD, a phenomenon that is hindering the diagnosis of CeD. Additionally, our strategy could be applicable to other disorders in which diagnosis in the absence of the disease-provoking insult is challenging, and is a good demonstration of the translation of the results of MR to the routine clinical practice. Funding: ISCIII 16/00258 and GVSAN2018/111086 to JRB.

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P16.50B
Gut microbiota abundances relate to gene methylation in blood in the Dutch LifeLines Deep cohort

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The gut microbiome is under investigation for its impact on human health. Since gene methylation can capture the long-term effects on gene regulation, its role as a determinant or target of gut microbial composition is of special interest. By integrating the population based data from the LifeLines-Deep cohort, we investigated the relations between gene methylation in blood and gut microbiota abundances. The microbiome data were generated by 16S (V4 region) sequencing of the DNA isolated from stool samples, and processed according to the MiBioGen pipeline available at https://github.com/alexa-kur/miQTL_cookbook. 271 taxa present in at least 10% of the individuals were selected. Methylation was detected by Illumina 450K methylation array, and dasen-normalized M-values were calculated. For each taxa we performed an epigenome-wide association study correcting for environmental, intrinsic and host genetic factors in the complete set of 702 individuals. Although no CpG-taxa pair reached experiment-wide significance calculated as false discovery rate, 49 pairs with P-value $< 2.4 \times 10^{-7}$ passed the significance threshold set for a single test. At genus level the most striking associations appear between *Lachnospiraceae sp*. abundance and methylation near gene *RAB18* (3.68×10^{-9}), *Coriobacteriaceae sp*. with *ARFGAP2* (1.86×10^{-9}) and *Bacteroides sp*. with *LRP3*. When integrating the shot-gun metagenomics data from the same samples, we found that *Bacteroides dorei* is the species responsible for the association with *LRP3* (6.15×10^{-7}). We demonstrate the potential impact of gut microbiome on the host epigenetics

and suggest candidate regulatory pathways which could be stimulated by distinct bacterial genera.

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P16.51C
Remus: a web application for prioritization of regulatory variants in monogenic diseases

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Introduction: Analysis of variants in distant regulatory elements could improve current 25-50% yield of genetic testing for monogenic diseases. However, the vast size of the regulome, great number of variants, and difficulty in predicting their phenotypic impact make searching pathogenic variants in the regulatory genome challenging. New tools for identification of regulatory variants based on their relevance to the phenotype are needed.

Methods: We used tissue-specific regulatory *loci* mapped by ENCODE and FANTOM5, together with microRNA - gene interactions from miRTarBase and miRWalk, to develop Remus - a system for identification of tissue-specific regulatory regions (https://github.com/seru71/remus). Remus combines regulatory features linked to known disease-associated genes, and filters them using activity status in target tissues. For user convenience, Remus provides a web interface and facilitates in-browser filtering of variant files, making it suitable for sensitive data.

Results: To evaluate our approach, we used a set of distal regulatory mutations reported causative for 23 distinct monogenic disorders, and a manually curated list of tissues affected by these disorders. Out of 23 mutated *loci* (enhancers and promoters) located 100bp-120,000bp away from the regulated genes, Remus correctly identified 19.

Conclusion: Remus facilitates identification of regulatory regions potentially associated with a monogenic disease, and can supplement classical analysis of coding variation with an aim of improving diagnostic yield of WGS experiments.

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P16.52D
An integrated bioinformatic pipeline for accurate and flexible DNA copy-number analysis from next-generation sequencing platforms

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Introduction: DNA copy-number variations (CNV) are a part of the human genome variability as well as associated with pathologies, including congenital genetic disorders and tumors. DNA-microarrays still dominate in CNV detection, however next-generation sequencing (NGS) is slowly taking over using coverage-based tools. In this study, we developed a comprehensive in-house bioinformatic pipeline that integrates CNV and loss of heterozygosity (LOH) analysis from various NGS-platforms and provides extensive annotation for routine use in research and diagnostics.

Methods: GATK4 tools were used to process bam files and data denoising. Allele-frequency (AF) data was obtained from gnomAD. CNV segments were called using the in-house algorithm and annotated by Variant Effect Predictor. Additional scripts were developed for AF denoising, visualization, QC and reporting. The pipeline was evaluated in two WES datasets (male infertility and primary immunodeficiency) and targeted-sequencing of cell line U266, focused on Ig-genes.

Results: Using the pipeline, we observed effective noise reduction even in bam files previously tested by other tools and displaying GC-wave. Independent validation showed that CNV detection was accurate, even within the Ig-loci

with complex rearrangements. Incorporating AF, we confirmed losses/gains and identified CN neutral LOH (cnnLOH) in addition to CNV. Our in-house segmentation offered the flexibility to adapt specificity and sensitivity, based on quality and coverage conditions. The visualization and data tables allowed reliable CNV interpretation.

Conclusions: We developed a robust and effective pipeline for CNV and cnnLOH detection. The pipeline is NGS-platform independent and can be adapted to match data segmentation, based on experimental conditions.

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P16.53A
Homozygosity mapping from small targeted NGS panels using SavvyHomozygosity - getting more from less

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Diagnosis of monogenic diabetes and hyperinsulinism is often achieved using targeted next generation sequencing (NGS). For consanguineous patients without a genetic diagnosis, the identification of regions of homozygosity can assist with both diagnosis and gene discovery. This is usually obtained using a microarray, or it can be calculated from genome sequencing data.

We aimed to develop a novel method to utilise the off-target reads from existing targeted NGS panel data to identify regions of homozygosity genome-wide.

We developed SavvyHomozygosity, which uses off-target reads from targeted NGS data in combination with linkage disequilibrium to calculate regions of homozygosity. We used data from 170 samples sequenced using both targeted NGS (average 3.4M reads per sample) and genome sequencing (mean read depth 35) to estimate the sensitivity and specificity of the method.

Regions of homozygosity larger than 3Mb were detected with sensitivity and specificity of 77%, and 10Mb with sensitivity and specificity of 93%. Detection was not limited to regions of the genome that are targeted by the sequencing panel.

SavvyHomozygosity identifies regions of homozygosity genome-wide in samples sequenced using a targeted NGS panel. The resulting data can be used to assist the discovery of causative variants, and can also be used with many samples to identify "hot spots" in the genome that may pinpoint an as yet undiscovered disease gene. The homozygosity data can also be used to identify candidate samples for more extensive sequencing.

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P16.54B
Analyses of olfactory related genes

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We study genes that participate in the olfactory pathway. We previously published RNAseq from human olfactory epithelium, a rarely studied tissue, and obtained a whole transcriptome overview. The results were incorporated into a study of 8 Israeli families with congenital general anosmia (CGA). Identified mutations in olfactory developmental genes suggest that the deficit results from abnormal embryonic development of the olfactory neuronal pathway.

In parallel, we study the evolution of the olfactory receptor genes (ORs), GPCRs with a crucial role in odor detection. The ORs undergo many species-specific duplications/deletions, resulting in complex orthology relationships. While for human a widely accepted nomenclature is available, based on phylogenetic classification into 18 families and further into subfamilies, for other mammals different nomenclature systems are used, concealing important evolutionary insights. We developed a systematic classifier for assigning a unified human-based nomenclature to any OR gene based on inter-species hierarchical pairwise similarities, and applied it to the OR repertoires of 7 mammals and zebrafish (10,247 ORs). This nomenclature generates a framework for evolutionary studies, where textual symbol comparison allows an immediate identification of potential orthologs and species-specific expansions/deletions, e.g. *Or52e5* and *Or52e5b* represent a duplication of *OR52E5* in rat. Another example is the absence of OR6Z subfamily among primate OR symbols. In other mammals, OR6Z members are disposed in one genomic cluster, suggesting a large deletion in the primate lineage.

This unified nomenclature is applied by the Vertebrate Gene Nomenclature Committee and its implementation is under consideration by relevant species-specific nomenclature committees.

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P16.56D

Systematic integration of genetic and epigenetic data yields novel insights into craniofacial development and orofacial clefting

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Craniofacial development (CD) is a complex embryonic process that shapes the human face. Defects in CD might lead to forms of orofacial clefting, such as nonsyndromic cleft lip with/without cleft palate (nsCL/P). Despite recent success by GWAS, the biological interpretation has largely been hampered by the lack of relevant tissue and the location of risk loci within non-coding regions. Here, we generated a new multi-ethnic meta-analysis on nsCL/P by combining three recent nsCL/P GWAS. Subsequently, these data were systematically integrated with epigenetic data from human neural crest cells (NCC) and embryonic facial tissue (eFT), covering the entire period of CD. In our meta-analysis, five novel risk loci were identified, increasing the number of nsCL/P risk loci to 45. For about 50%, available data on histone modifications and chromatin accessibility revealed distinct epigenetic activity patterns in NCC and eFT. The Combination with interaction data revealed novel candidate genes with yet unknown roles in CD. For instance, at the 1p22 locus, we confirm the presence of active enhancers within *ABCA4* that regulate *ARHGAP29* expression. However, our data also suggest that another gene, *ABCD3*, becomes activated at later stages of CD. This first systematic integration of functional data from relevant embryonic cell types provides new insights into the etiology of nsCL/P and the biological impact of associated variants. Our data indicate that different genes become activated and repressed in a time- and tissue-specific manner, which implies that the "one locus - one candidate gene" theory might be too simplistic, at least for nsCL/P.

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P16.57A

When the outlier is the signal: Denoising autoencoders to pinpoint causes of rare diseases from RNA-seq data

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For most individuals with a suspected rare Mendelian disorder, no clear pathogenic variant can be pinpointed after undergoing genome sequencing. A promising complementary avenue is to identify causes of rare diseases as aberrant regulatory events (RNA level or splicing) in RNA sequencing data. However, existing methods for detecting aberrant regulatory events in RNA-seq data either lack assessments of statistical significance or rely on subjective manual corrections for confounders. Here, we address these issues by using denoising autoencoders as generic tool to detect outliers while automatically controlling for co-variation resulting from technical, environmental, or common genetic variations. Our model to detect expression outliers, implemented in the Bioconductor package OUTRIDER, uses the negative binomial distribution to model RNA-seq read counts. OUTRIDER improves upon state-of-the-art expression outlier methods for detecting simulated outliers, genes with rare variants possibly causing non-sense mediated decay in the GTEx dataset, and pathogenic rare expression events in a rare disease cohort. We will also present unpublished results on splicing outlier detection based on the beta-binomial distribution. We use an annotation-free approach to detect novel splice sites, and consider both exon-exon junction reads and reads overlapping exon-intron boundaries to detect intron retention events. Application to a mitochondrial disease cohort demonstrated increased sensitivity over our previous splicing outlier method and led to a new diagnostic by

identifying an aberrant exon truncation in the gene *TAZ*. Altogether, these results indicate that employing denoising autoencoders may become a general strategy to identify causes of rare diseases in omics data.

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P16.58B
Guidelines for the detection of somatic mutations from whole exome sequencing data: a case study of Parkinson's disease patients

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Introduction: Accurate calling of somatic mutations from bulk sequencing data still poses a challenge outside the scenario of clonal expansion in cancer. Low frequency mutations present in a tissue have been shown to exist in other tissues, even of different germ layer origin. Consequently, defining tissue-specific variants is an inefficient strategy for detecting these mutations, which increases the need to identify the confounding factors hampering the discrimination of true somatic variants from noise. As a case study, we assessed the detection of somatic mutations in sporadic Parkinson's disease (PD).

Methods: We sequenced the whole exomes of substantia nigra, striatum, neocortex, cerebellum and peripheral blood from 10 PD patients. Confidence of detected variants was assessed by comparative analyses.

Results: Inter-tissue and inter-individual comparisons showed high variability of variant allele frequency (VAF) even for high confidence heterozygous variants (21.1% of calls with a VAF <0.4 or >0.6). Moreover, we found that on-target calls VAFs are significantly shifted towards the reference allele, demonstrating a probe bias in exome sequencing data relevant for this application. Remarkably, we also observed that alignment artifacts derived from unresolved regions still emerge even after removing genomic regions difficult to align, such as low mappability regions or segmental duplications. Based on these observations, we propose a series of guidelines to call somatic mutations and describe a set of high confidence calls from 10 PD patients.

Conclusions: The use of multiple tissues or replicates as well as awareness of the error sources are of great importance to call somatic mutations.

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P16.59C
The gut microbiome of Kashmiri women with polycystic ovary syndrome shows significant enrichment at multiple genera, including Bifidobacterium

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Polycystic ovary syndrome (PCOS) is a common endocrine condition in women of reproductive age characterized by polycystic ovaries, hyperandrogenism, oligo- or amenorrhea, and a constellation of metabolic derangements. Gut microbiome composition might contribute to PCOS susceptibility. We profiled the microbiome in DNA isolated from faecal samples by 16S rRNA sequencing of 19/20 Kashmiri (India) women with/without PCOS. We excluded bacteria not detected in at least 1/3 of the subjects or with less than 0.1% average relative abundance. We compared the relative abundances of 40/58 operational taxonomic units in family/genus level between cases and controls, and in relation to 33 hormonal and metabolic factors, by multivariate analyses adjusted for stool consistency, day of menstrual cycle at sample collection, sequencing read depth and age, and corrected for multiple testing. For the first time, we detected a positive significant link between the butyrate production-related *Eubacterium* and follicle-stimulating hormone levels. We identified significant enrichment in *Bifidobacteriaceae* (median 6.07% vs. 2.77%) and *Aerococcaceae* (0.03% vs. 0.004%), whereas we detected lower *Peptococcaceae* levels (0.16% vs. 0.25%) in PCOS cases. Additionally, seven genera were significantly enriched in PCOS cases: *Sarcina, Alkalibacterium* and *Megasphaera*, and the previously PCOS-associated *Bifidobacterium, Collinsella*, *Paraprevotella*

and *Lactobacillus*. We observed enrichment of *Collinsella* and *Paraprevotella* with higher fasting blood glucose levels, and *Paraprevotella* and *Alkalibacterium* with larger hip and waist circumference, and weight. We show a relationship between gut microbiome composition and PCOS, and link it to specific metabolic and hormonal predictors of reproductive health in Indian women.

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P16.60D
Genetic clusters and population stratification in the Estonian Biobank and its association with complex traits

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Introduction: It has been shown that principal components (PCs) mainly indicate individuals' geographic ancestry. Therefore, the PCs have often been used for adjusting genome-wide association analyses for population stratification. It can be assumed that this is particularly relevant in a genetically heterogeneous population. Large-scale population-based biobanks have increased the potential to study the impact of fine-scale stratification and cryptic relatedness also in small and homogeneous populations.

Methods: We analysed 49,363 participants from the EGCUT cohort genotyped using various Illumina arrays. The individuals are sampled from all counties of Estonia. We calculated the first 250 PCs using 61,044 independent variants.

Results: As most of the first 250 PCs were significantly associated with the county of birth, we show that it is most efficient to use all of them in the k-means algorithm. Next, we tested the association of cluster membership, actual county of birth as well as the individual PCs with prevalent coronary heart disease (CAD) and type 2 diabetes (T2D), and polygenic risk scores (GRS) for the same diseases. Three first PCs as well as county of birth were significantly associated with prevalent CAD status, but not with T2D. The GRS for T2D was significantly associated with the first eleven PCs and the GRS for CAD with first and third PC, but both GRSs were associated with the cluster membership.

Conclusions: Our results indicate that although PCs may help to adjust for population stratification, a genetic cluster indicator may be more efficient in capturing the confounding due to geographic variation.

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P16.61A
GENETIC DETERMINANTS OF TRANSCRIPTIONAL VARIATION IN PRIMARY HUMAN MONOCYTES ACROSS MULTIPLE CONTEXTS

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Inter-individual variation in the regulation of gene expression is a key driver of phenotypic diversity. Monocytes form sentinel cells within the innate immune system and are implicated in numerous inflammatory disease processes. We have previously found a significant proportion of monocyte eQTL to be detectable only after activation. The degree to which activation state modulates genetically determined gene-splicing events and differential transcript usage is unclear however. Here we use 100bp paired-end RNA-sequencing to systematically explore genetic determinants of gene-expression and isoform usage across monocytes from 192 individuals in the naive state and post-exposure to IFNγ or lipopolysaccharide (LPS). We observe novel splicing effects and unappreciated complexity at many loci associated with a high degree of chromatin accessibility. We similarly replicate and extend previous observations of *trans* acting master-regulatory regions (e.g. *NFE2L3*, *IRF2*, *IFNB1*). Specifically, we identify 7,275 lead eQTL for 7,631 transcripts. We find isoform QTL show a high degree of context specificity, with the majority being unique to one treatment state. We apply an empirical Bayesian approach to model context-specific eQTL and facilitate the identification and prioritization of the most likely causal variants. We integrate these results with genomic regulatory features, chromatin interaction datasets, and summary-statistics from 761 UK-Biobank traits to further our understanding of the biological role of context-eQTL. Our findings provide novel insights into the context-

specific role of genetic effects in the regulation of the transcriptional machinery in primary immune cells and identify new potential insights into complex disease processes.

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P16.62B

Whole platelet transcriptome profiling confirms a cytoskeleton remodeling in platelet components associated with adverse transfusion reactions

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Introduction: Platelets are small anucleate cells that play a key role in hemostasis and thrombosis. Blood platelets destined for the transfusion release panoply of molecules during preparation and storage. The leukoreduction process made the transfusion safer but did not abolish the adverse events. The purpose of this work was to study the whole platelet transcriptome when found in association with adverse transfusion reactions (ATRs).

Methods: Total RNA isolated from six buffy-coat-derived pooled platelet components (PPC) were sequenced using the Ion-Proton® technology. The alignment of the reads to the hg19 reference genome were done with STAR software. The genes were quantified, using the EM algorithm method implemented in Partek Flow® software. DESeq2, Edge R and Limma voom (three R packages) were used for differentially expressed (DE) gene identification. Gene-Gene interactions and clustering analyses were performed with STRING db to identify hubs genes and clusters of interests. PANTHER allowed performing the functional enrichment analysis.

Results: Among 79 dysregulated DE genes (common with the 3 methods), 6 transcripts (score confidence: 90%) encoding proteins were found to be related to the dysregulated network of the cytoskeleton remodeling, associated with a cellular reorganization. RhoC was strongly present, with DE genes involved in the signaling of chemokines.

Conclusion: Platelets in PC having led to clinical ATRs in the transfused patients were found to exhibit profound transcriptome changes. This study contributes to better understand the pathophysiological aspect of ATRs that appear to be associated with the modification of the platelet cytoskeleton. Grant EFS- APR-2016-32, ART-INTS, "Les-Amis-de-Rémi", France.

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P16.63C

Finding the diagnosis for rare disease patients in southern Sweden by coordination of high-resolution genomic arrays (CytoScan HD/XON, Thermo Fisher Scientific) and massive parallel sequencing (CREv2, Agilent/NextSeq500, Illumina)

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Introduction: Until recently, it was difficult to identify genetic causes of heterogeneous diseases and syndromes. Today, advances in two crucial techniques, high resolution genomic arrays and massive parallel sequencing, makes it possible to investigate essentially all genes in parallel. A consensus has been established that parallel or sequential genomic array and exome analysis, should be considered as first-line tests in the evaluation of children with *e.g.*, unexplained intellectual disability and/or congenital anomalies.

Methods and Results: The main objective of the presented workflow is to provide a smooth and financially viable pipeline to diagnose rare disease patients in southern Sweden by combining genome wide copy number detection and innovative SNP/UPD-analysis of genomic arrays with exome sequencing. The presented results and analysis pipeline, including regionally developed bioinformatic and visualization tools, are based on comprehensive clinical validation and experience from 9 years of genomic array and 4 years of exome sequencing. A complementary

method, CytoScan XON, was implemented in 2018 to detect copy number variants down to 1 exon.

Conclusions: We conclude that genomic arrays and exome sequencing are broad and powerful methods which complement each other, and we believe these methods provide a strong foundation throughout the foreseen transition towards whole genome sequencing. We further conclude that, in a clinical setting, together with careful standardized phenotyping and local expert interpretation and bioinformatic teams, the described workflow can deliver a diagnostic yield of >50% at a sustainable cost to the regional health care system.

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P16.64D
Detecting pathogenic repeat expansions from genome sequence data

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Repeat expansions are an important class of genetic variation in neurological diseases and may represent a convergent aetiological molecular mechanism. However, the identification of novel repeat expansions using conventional sequencing methods is a challenge due to their typical lengths relative to short sequence reads and difficulty in producing accurate and unique alignments for repetitive sequence. However, this latter property can be harnessed when using paired-end short read sequencing data to infer the possible locations of repeat expansions and other structural variation.

Here we present REscan, a fast and lightweight command line utility that infers the possible locations of repeat expansions from paired-end short read sequencing data by reporting the proportion of reads orientated towards a locus that do not have an adequately mapped mate. A high number for this statistic relative to a population of data indicates the location of a possible repeat expansion that can be cross-referenced against structural variant calls derived from alternative algorithms and brought forward for experimental follow-up. We validate this approach using whole-genome sequence data for 259 cases of amyotrophic lateral sclerosis, of which 25 are positive for a large hexanucleotide repeat expansion in *C9orf72*, determined by repeat-primed PCR. We show that REscan has good discriminative accuracy in identifying repeat expansions from paired-end sequence data, and application genome-wide can be used in an initial screen to infer the locations of other repeat expansions, thus accelerating the discovery of novel disease-relevant genetic variation.

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P16.65A
SPiP: a Splicing Prediction Pipeline addressing the diversity of splice alterations, validated on a curated diagnostic set of 2,468 exonic and intronic variants

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Variant interpretation is recognized as the major challenge in genetic diagnosis. Spliceogenic variants exemplify this issue as all types of nucleotide variations can be pathogenic by affecting normal pre-mRNA splicing via disruption/creation of splicing signals such as splice sites (ss),

branchpoints (BPs) or splicing regulatory elements (SREs). Unfortunately, most in silico prediction tools are dedicated to specific signals (eg 5'/3'ss, BPs or SREs). We developed the Splicing Prediction Pipeline (SPiP) to allow comprehensive assessment of variant effect on the different regulatory motifs involved in splicing. SPiP runs a cascade of different and complementary tools, chosen on their efficiency, *i.e*. Splicing Prediction in Consensus Element (SPiCE) for physiological 5'/3'ss, Branch Point Prediction (BPP) for BPs, ΔtESRseq for SREs. Moreover, we embedded a new score for the prediction of cryptic/*de novo* ss, after training and validation on more than 200 million of ss obtained through Ensembl data. SPiP was evaluated on a curated diagnostic collection of 2,468 variants (19.8% unpublished) in 345 genes, with their corresponding experimental RNA splicing data. These variants were scattered along the exonic and intronic sequences up to 36,947 bp from the ss. SPiP achieved an accuracy of 80.5 %, with a specificity of 71.3 % and a sensitivity of 91.07 %. As a result, SPiP is a comprehensive prediction pipeline which properly deals with the diversity of possible splicing alterations. It can be easily implemented in any diagnostic laboratory as a routine decision making tool for prioritizing RNA studies.

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P16.66B
PrimerBench: A modern framework for continuous primer validation

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Introduction: The usage of primers of Sanger sequencing is essential in molecular diagnostics. The quality of those primers is critical for a good analysis of the targeted genomic regions during sequencing. The quality of a primer depends on many factors, as for example, the absence of variants on the region covered by the primers themselves. A primer with variants would fail to amplify both alleles and consequently lead to allele dropout in the sequencing results. Given the large amount of primers managed by a laboratory and their quality depending on continually updated data, an automated reassessment is needed.

Methods: A web application has been developed using python and the Django web framework. To determine the location of the primers on the reference genome we use Blast. The variants present at the location of the primers is recovered through the Ensembl REST API. We implemented configurable validation checks for the primers, such as a maximum MAF for any variant found inside the primer. Primers can be reanalyzed at any time to take into consideration updated external databases.

Results: We developed an open-source web based solution to validate primers, accessible at https://genetic.tools. Users can be organized in laboratories, sharing primers and validating them whenever public databases such as Gnomad, TOPMed or others update their variant information, or the reference genome is updated. Those updates can be automated, with automatic alerts to the users if the updated information on their primers.

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P16.67C
A SMARTer solution to stranded single-cell RNA-seq

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Introduction: With the growing need for low-input and single-cell NGS library prep solutions, we see that researchers recognize the value in revealing transcriptome profiles from damaged cells as well as noncoding information from extremely low cell numbers (1-1,000). We have previously released several RNA-seq solutions based on oligo(d)T priming that push the limits of sensitivity and reproducibility from ultra-low inputs as well as single cells. Oligo(d)T priming is a very efficient way to capture the transcriptome, only the polyadenylated fraction can be captured. In addition, for oligo(dT)-primed cDNA synthesis to generate high-quality libraries, one needs to start with high-quality RNA, which excludes the use of this technology with samples damaged or degraded due to the nature of the processing (e.g., FFPE samples) or method of isolation. Additionally, these earlier single-cell kits do not preserve strand-of origin information.

Methods and Results: SMART-Seq Stranded Kit now allows the generation of sequencing-ready, stranded Illumina® libraries directly from 1-1,000 sorted cells or an

equivalent amount (10 pg-10 ng) of purified total RNA of any quality. This kit integrates an innovative technology, already incorporated in our SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian, which enables removal of ribosomal cDNA following cDNA synthesis, as opposed to direct removal of corresponding rRNA molecules prior to reverse transcription. The SMART-Seq Stranded Kit protocol can be completed within seven hours, and a convenient pooling option for inputs between one to ten cells facilitates greater ease-of-use by minimizing the number of samples being handled.

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P16.69A
PopDel calls medium-size deletions jointly in tens of thousands of genomes

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Catalogs of genetic variation for large numbers of individuals are a foundation for research on human diversity and disease. Creating such catalogs for small variants from whole-genome sequencing (WGS) data is now commonly done with joint calling approaches. We have transferred the joint calling idea to larger deletion variants and developed the first joint calling tool, PopDel, that can detect deletions in WGS data of tens of thousands of individuals simultaneously. In the joint analysis of hundreds of simulated genomes, PopDel shows similar recall and precision to existing deletion callers while being faster and requiring less memory. On data of the extensively studied genome NA12878, PopDel identifies more deletions contained in GiaB and PacBio reference call sets compared to its competitors Delly and Lumpy. On Illumina Polaris data, PopDel's deletions distinguish individuals from different populations indicating that the callset reflects biological differences. In PopDel's output for 49 Polaris trios, we identified a *de novo* deletion in the individual HG01763 confirmed by multiple SNPs that further indicate its origin on a maternal haplotype. Application to WGS data of tens of thousands of Icelanders including 778 Icelandic trios yields a preliminary Mendelian inheritance error rate of 0.05% (28,210,207 trio-deletion-pairs) using only a filter on genotype likelihoods. Based on these results, we believe that PopDel will enable more routine scans for deletions in large sequencing cohorts.

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P16.70B
Enrichment and Clustering of Rare Genetic Variants using Shared Protein Structure Domains

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Introduction: The spatial distribution of variants in proteins can suggest mechanisms of disruption and can help to differentiate benign from damaging changes. However, in rare diseases the sparsity of variants per protein reduces the power of spatial distribution analysis. We overcome this by enriching rare variant data and aligning shared structural domains from different proteins. We then analyse the domains together and uncover spatial patterns using DBSCAN 3D clustering.

Materials and Methods: The variants are mapped from DNA coordinates to the position on the most appropriate protein structure. They are then grouped by CATH structural domain and protein structures within each domain containing a variant are aligned. DBSCAN clustering is then performed on the 3D coordinates of the variants in the aligned structure space for each CATH domain family.

Results: Firstly, comparing the locations of clusters with those of known disease-associated variants and those considered benign can help to assign a pathogenicity probability to variants of unknown significance. Secondly, the location of the cluster in the protein or complex can suggest potential mechanisms of action such as the interruption of ligand binding, catalysis or structure destabilization/misfolding. Thirdly by comparing members of the same spatial clusters with gene lists of known disease association we can uncover new disease gene candidates.

Conclusion: Enrichment and spatial clustering of rare variants on protein structures can allow disease-sensitive regions of proteins to be uncovered, new disease associated genes to be found and can help to characterise variants of unknown significance.

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P16.71C
Using multiple sequencing platforms to identify and characterise disease-causing genome alterations

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Genomic alterations, including single nucleotide polymorphisms (SNPs), small insertions/deletions (InDels) and structural variants (SVs) are a major determinant of congenital disease. Whereas coding SNPs and InDels have been the focus of intense study, and rare small variants have been implicated in about 40% of rare genetic developmental disorder diagnoses, little is known about the mechanisms and disease impact underlying non-coding variation. Non coding SNPs can affect regulatory elements and have been causally linked to Mendelian disorders. SVs can alter the genome by duplicating, deleting, inverting or translocating genomic segments. SVs, in particular, are poorly understood, mainly because of the difficulties inherent with their reliable detection and phasing via short read sequencing technology. Genomic alterations that remain cryptic to current sequencing technology are likely to represent a significant source of disease-causing variation in unsolved Mendelian disorders.

Here, we aim to comprehensively discover, phase and characterise the spectrum of genomic variation for a cohort of several individuals affected by rare congenital disorders, using an array of genomic resources: deep short read whole genome sequencing (WGS), in situ Hi-C, long read sequencing (PacBio), optical mapping (Bionano) and linked-read (10x Genomics) technologies.

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P16.74B
Identification of uniparental disomy events in a cohort of 29,723 whole exome sequencing samples

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Introduction: Uniparental disomy (UPD) is the occurrence of two homologous chromosomes, or segments originating from the same parent. Although the majority of UPDs does not have phenotypic consequences, particular events may lead to disease due to imprinting effects, an underlying homozygous pathogenic disease variant or a low mosaic trisomy of the respective chromosome. UPD were typically identified by SNP microarrays during routine genetic diagnostics. Here we present a validated method to detect UPDs in both trio and single whole exome sequencing (WES) data.

Material and Methods: We applied UPDio for the detection of UPD based on Mendelian inheritance errors to a cohort of 4,912 WES trios and used identified regions of homozygosity (ROH) by H3M2 for isodisomy UPD (iUPD) detection to a cohort of 29,723 single WES samples. We calculated per chromosome whether the ROH size deviated significantly from the log-normal distribution and we reported cases with ROH involving a whole chromosome.

Results: Among the 4,912 WES trios we identified 9 (0.18%) UPD events, 3 of which involved isodisomy and 6 heterodisomy. In the cohort of 29,723 single cases we found 13 (0.04%) iUPD events, including the 3 found in trios. At least 11/22 UPD findings had previously been reported. Two involved a known imprinted chromosome disorder and in at least four patients a homozygously mutated disease gene was identified.

Conclusions: UPD can be identified using both single and trio WES. UPD can be clinically relevant and may affect genetic counseling, given the reduced risk of recurrence for affected families.

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P16.75C
An ultrafast amplicon-based targeted library preparation method using double-stranded unique molecular identifiers for detecting rare mutations in cell-free DNA

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Introduction: Liquid biopsy is a noninvasive easily obtainable sample with diagnostic value, especially using cell-free DNA (cfDNA). But it presents challenges toward accurate variant detection, as low fractions of mutant DNA present can be masked by artifacts or background noise from PCR and sequencing errors, leading to false negatives. Progress has been made using hybrid capture-based

sequencing methods using unique molecular identifiers (UMIs), but can be time-consuming with complicated and tedious workflows, resulting in poor results. Large amounts of cfDNA for these protocols are not realistic to obtain from patients. To provide a fast (4-hour) and reliable NGS solution for low-frequency variant detection, we developed the CleanPlex® UMI technology.

Methods: CleanPlex® UMI technology features 3 steps to generate molecular-barcoded NGS libraries: multiplex PCR for molecular barcoding, background cleaning step to remove PCR by-products, and indexing PCR to add Illumina® adapters and sample indexes. Here we present a panel targeting frequently mutated hotspots in 23 genes in lung cancer. Libraries were prepared with reference cfDNA (SeraCare) at 0.1% to 0.5% minor allele frequencies (mAF) and sequenced using Illumina® NextSeq®.

Results: With CleanPlex UMI Lung Cancer panel at input of 50ng cfDNA nearly all mutations are detected at 0.1% mAF and at 0.25% mAF with 100% PPV. With input of 20 ng of cfDNA, the method can achieve 100% detection at 0.5% mAF.

Conclusion: The CleanPlex® UMI technology demonstrates high sensitivity with low false positive rate, for the detection of low-frequency alleles, even at low DNA inputs with an easy cost-effective workflow.

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P16.77A
Combining ACMG guideline and multiple public databases identifies misclassified HGMD variants

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Background: Discordant variant classifications between public databases is one of the well-documented limitations when interpreting pathogenicity of variants. The aim of this study is to investigate the level of variant misclassification from the Human Gene Mutation Database (HGMD) and the annotation concordance between databases in depth.

Materials and Methods: We used a total of 166,834 classified variants [disease-causing mutations (DM, n=157,874) and disease-associated polymorphisms (DP, n=3,275; DFP, n=2,009; FP, n=3,676)] from the HGMD. All variants were reanalyzed based on the American College of Medical Genetics and Genomics (ACMG) guideline and compared to ClinVar variants. Misclassification types were categorized into major discordance (from pathogenic to benign or vice versa) and minor discordance (from pathogenic to variants of unknown significance or vice versa).

Results: According to variant classification based on ACMG guideline, major discordance were observed in 0.1% (559/157,874) of DMs and 6.2% (129/3,676) of polymorphisms. Major discordance was frequently observed in nonsense (41.3%), splicing (10.6%), or frameshift (29.8%) variants, while minor discordance was occurred in nonsynonymous variants (84.9%). Overall concordance between HGMD and ClinVar were 89.9% (39,792/44,772) variants studied. The remaining 10.1% of variants was discordant classification, most of which are nonsynonymous variants.

Conclusions: Variant classification from databases needs to use with caution when interpreting pathogenicity of variants. We found that significant number of loss of function variants, which was reported pathogenic in databases may not be pathogenic. This is the first study to reveal misclassification burden of the HGMD variants and annotation concordance at the genome-wide level.

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P16.78B
Loqusdb: the value of a local frequency database

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Variant frequencies are essential when differing between benign and potentially disease causing variants in rare disease cases. After analysis and annotation of exomes and whole genomes we are presented with thousands of candidate variants that calls for further investigation. One of the most effective and unbiased way to reduce this number is to assess the rarity of a variant in any population. This approach is especially powerful when working in the field of rare diseases. Currently there are a number of reliable sources of information for major population frequencies when considering single nucleotide variants (SNV) and small indels, with gnomAD as the most prominent public resource available. In contrast, for structural variation (SV), the background frequency in the general population is more or less unknown mostly due to challenges in calling SVs in a consistent way. Keeping track of local variation can reduce the number of potential disease causing variants, both for SNVs and SVs, in a considerable way. Here, we present **loqusdb**, a tool to solve the problem of keeping track of any type of variant frequencies. Loqusdb was designed to handle a large flow of samples and unlike other solutions samples can be added continuously to the database without rebuilding which simplifies the situation. We show both how powerful SV analysis can be when considering population frequencies and also how the number of SNV/INDELS can be reduced by adding local population frequency information even after annotating with gnomAD.

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P16.79C
Quantitative approaches to variant classification increase the yield and precision of genetic testing in Mendelian diseases

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Guidelines for variant interpretation in Mendelian disease set stringent criteria to report a variant as (likely) pathogenic, prioritising control of false positive rate over test sensitivity and diagnostic yield. For genetically heterogeneous diseases where missense variants constitute the preponderance of disease-causing variants, this leads to high rates of variants of uncertain significance in known disease genes and false negative test results, as many variants detected in patients will be novel or poorly characterised.

We compared rare variants in large case cohorts of inherited cardiac conditions to reference populations to identify variant classes with high prior likelihoods of pathogenicity, defined by etiological fraction (EF). Analysis of variant distribution using a bespoke unsupervised clustering algorithm identified gene regions where case variants are significantly clustered. Non-truncating variant categories with EF≥0.95 were identified in 5 established hypertrophic cardiomyopathy (HCM) genes and high EF regions defined in major arrhythmia genes (*RYR2*/*SCN5A*). We propose adaptations to ACMG/AMP guidelines to incorporate this quantitative evidence and demonstrate substantial increases in diagnostic rates, e.g. a 14-20% relative increase in HCM cases with actionable variants.

Particular variants classes are empirically shown to have a sufficiently high probability of pathogenicity to support a "likely pathogenic" classification when found in patients, even without segregation or functional evidence. Our proposed adaptations to ACMG/AMP guidelines can be seamlessly incorporated into clinical genetics laboratory workflows for diseases with large genetic datasets. This approach could increase the yield of high confidence actionable variants in many Mendelian diseases, consistent with the framework and recommendations of current guidelines.

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P16.80D
An Artificial Intelligence Engine for High-Throughput Matching of Genetic Variants to their ACMG/AMP Classification for Inherited Disease Gene Panels

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The ACMG/AMP evidence-based guidelines for variant pathogenicity assessment define several criteria assessing particular supporting evidence information. Criteria are combined to classify a variant as either pathogenic (P), likely-pathogenic (LP), benign (B), likely-benign (LB), or uncertain significance (VUS). Although widely adopted in clinical interpretation of variants this process has remained largely manual and time-consuming. Current informatics tools aimed to ease the application of the guidelines do not completely automate the entire process. Therefore, we developed an artificial intelligence method to automatically infer the classification of variants that utilizes a forward-chaining inference engine at its core to implement the ACMG-AMP criteria returning a predicted classification for each variant. The engine is able to incorporate criteria and rule refinements for specific genes by separating the rule specification from the code that executes them. Natural language generation provides explanatory text with the rationale of the classification attained for reference by clinical geneticists. To evaluate the performance of our method, we analyzed a set of 19,990 variants for a 15-gene hereditary breast and ovarian cancer panel with prior classifications from ClinVar (2+ stars) and Color Genomics database used as ground truth. We show automatic classification of 91.6% of P/LP and 60% of B/LB ClinVar variants with essentially no misclassifications. Unclassified variants are annotated with resolved guideline criteria for rapid manual assessment to finalize classification. In addition, we show the ability to reclassify 76% previously VUS variants of the Color database. We are integrating this engine into our cloud-based software platform for clinical genomic interpretation.

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P16.81A
Enhanced access to extensive phenotype and disease annotation of genes and genetic variation in Ensembl

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The accurate annotation and interpretation of genes and genetic variation is paramount in basic research and clinical diagnostics. Critical information such as population allele frequencies, the predicted molecular consequences of variants and observed associations with phenotype often lie in decentralized large-scale resources and are time consuming to integrate.

We import and harmonize phenotype and disease annotations from sources including ClinVar, DGVa/dbVar, the NHGRI-EBI GWAS Catalog, DDG2P, Orphanet, MIM morbid and the Cancer Gene Census into a standard structure and provide simple tools for data access.

To facilitate improved querying across conditions described differently in different studies and support aggregated views in the Ensembl genome browser, we map the phenotype descriptions used in these projects to ontology terms. We have recently updated the Ensembl Variant Effect Predictor (VEP) to provide enhanced phenotype and disease annotations alongside predicted molecular consequence and frequency data. We have also recently extended our REST API to support detailed querying of these data.

In Ensembl release 96 (April 2019) we have 54,859 human phenotype/disease annotations associated with genes and 5,910,851 associated with variants from 15 different sources. These can be extracted using a variety of interfaces and are available for variant annotation with VEP.

We import an extensive range of phenotype and disease annotations into one unified resource. Here we describe the data types we hold and the multiple access methods we have developed to simplify the use of these essential data within the community.

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P16.82B
Implementation of WGS in diagnosis through extensive computational optimization

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Whole Genome Sequencing (WGS) is becoming the standard of care to diagnose rare hereditary diseases as it promises to deliver more information than the current standard of care, namely arrayCGH and whole exome sequencing (WES). Its implementation in a diagnostic setting will homogenize the molecular laboratory workflow, offer a single test for multiple conditions, and may ultimately decrease cost and time to diagnosis. Although the bioinformatics pipelines used for analyzing WGS and WES are very similar, implementing WGS analysis in routine diagnosis is hampered by the huge amount of data generated. In the ICON-GAP project, we developed and evaluated new solutions for WGS data analysis. First, we implemented GATK Base Quality Score Recalibration in the new version of elPrep, a high-performance tool for preparing BAM files for variant calling. The use of elPrep to sort BAM files, remove duplicates and perform base quality score recalibration produces the same output as the traditional samtools/Picard/GATK approach but runs seven times faster on WGS data. Second, we integrated this version of elPrep in Halvade, a framework for executing pipelines in parallel. Halvade's efficient parallelization enabled to complete the analysis of a 30X WGS in three hours. Finally, we compared the results obtained using elPrep and Halvade to those obtained using the Broad Institute production pipeline run on the Google Genomics cloud using well-characterized cell lines. In conclusion, we developed and optimized a software infrastructure that significantly minimizes the computational needs for WGS, hence enabling the short-term implementation of WGS into full diagnostic use.

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P16.83C
VarFish - fishing for causative variants

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VarFish is an easy-to-use web-based database system designed for empowering geneticists in the analysis of clinical and whole exome sequencing variant data sets for individuals and families. It provides a set of tools for supporting the full workflow from (i) variant data quality control, (ii) variant filtration and (iii) efficient assessment of variants based on visual alignment inspection and annotation data such as functional and frequency annotation.

The system allows to organize data into a project folder structure with access control. Variant quality metrics can be displayed project- (and thus cohort-) wise or for single cases/families. The variants themselves can be filtered based on genotype, population frequency, variant effect, quality metrics, and annotation such as membership in ClinVar or HGMD Public. A special ClinVar-centric view allows for the easy screening of variants based on pathogenicity annotation in ClinVar.

After filtration of the variants, their quick and efficient assessment is supported by various tools: color flags and commenting allows for simple yet effective note-taking, remote-controlling the genome viewer IGV to display a variant's locus, and some important database excerpts are available directly within the system. Further, link-out to external databases (e.g., ENSEMBL or NCBI), allow the assessment of variants using MutationTaster, SIFT, or various splice site assessment tools. Filtered data sets can be downloaded as VCF or Excel files (including comments and flags) or submitted to external tools such as MutationDistiller.

VarFish is available under a permissive open source license and ships with comprehensive documentation and installation instructions.

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P16.84D
Detecting and sizing short tandem repeat expansion mutations in whole genome sequence data

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Introduction: Whole genome sequencing (WGS) has unprecedented potential to identify disease-causing genetic variants. However, most clinical WGS bioinformatic pipelines overlook variations in short tandem repeat (STR) regions, which constitute ~3% of our genomes. Several algorithms to analyze STRs in WGS data have been developed recently, yet a comprehensive characterization of their reliability in detecting and sizing STR expansions is lacking.

Methods: We compared the performance of eight available STR algorithms (lobSTR, RepeatSeq, HipSTR, TREDPARSE, ExpansionHunter, STRetch, exSTRa and GangSTR) on the European Genome-phenome Archive dataset of 118 Coriell genomes with known repeat expansion mutations in one of eight different disease-associated STR loci. We evaluated the sensitivity of the tools in detecting the disease-associated full-mutation (FM) expansions in this gold standard dataset and ascertained the concordance between estimated and actual repeat lengths.

Results: lobSTR, RepeatSeq and HipSTR failed to detect STR expansions altogether, while TREDPARSE, ExpansionHunter, STRetch, exSTRa and GangSTR exhibited 100% sensitivity in detecting FMs at *AR* and *ATN1* loci, and identified FMs in 94-100%, 85-100% and 50-100% of *DMPK*, *HTT* and *ATXN1* expansion-positive samples, respectively. Only ExpansionHunter, GangSTR and STRetch identified the single *ATXN3* FM sample, whereas none of the algorithms reliably detected *FMR1* FMs. TREDPARSE and GangSTR did not identify the homozygous *FXN* FM expansions, while STRetch, ExpansionHunter and exSTRa exhibited sensitivities of 79%, 57% and 29%, respectively. While most callers appeared to perform well in sizing non-expanded alleles, ExpansionHunter's repeat size estimates, in general, were closer to the actual lengths of both non-expanded and expanded STR alleles.

I. Rajan Babu: None. **R. Chiu:** None. **M. Couse:** None. **I. Birol:** None. **J. Friedman:** None.

P16.85A

Comprehensive variant detection in a human genome with highly accurate long reads

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Introduction: Long-read sequencing has been applied successfully to assemble genomes and detect structural variants. However, due to high raw-read error rates (10-15%), it has remained difficult to call small variants from long reads. Recent improvements in library preparation and sequencing chemistry have increased length, accuracy, and throughput of PacBio circular consensus sequencing (CCS) reads, resulting in 10-20kb reads with average read quality above 99%.

Materials and Methods: We sequenced a 12kb library from human reference sample HG002 to 18-fold coverage on the PacBio Sequel II System with three SMRT Cells 8M. The CCS algorithm was used to generate highly-accurate (average 99.8%) 11.4kb reads, which were mapped to the hg19 reference with pbmm2. We detected small variants using Google DeepVariant with a model trained for CCS and phased the variants using WhatsHap. Structural variants were detected with pbsv. Variant calls were evaluated against Genome in a Bottle (GIAB) benchmarks.

Results: With these reads, DeepVariant achieves SNP and Indel F1 scores of 99.82% and 96.70% against the GIAB truth set, and pbsv achieves 95.94% recall on structural variants longer than 50bp. Using WhatsHap, small variants were phased into haplotype blocks with 105kb N50. The improved mappability of long reads allows us to align to and detect variants in medically relevant genes such as *CYP2D6* and *PMS2* that have proven "difficult-to-map" with short reads.

Conclusions: These highly-accurate long reads combine the mappability and ability to detect structural variants of long reads with the accuracy and ability to detect small variants of short reads.

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P17
Epigenetics - Gene regulation

P17.01B
Methylome-wide association study of amyotrophic lateral sclerosis case-control cohorts

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Amyotrophic lateral sclerosis (ALS) is the most common subtype of the motor neuron diseases. The clinical heterogeneity of ALS may reflect a complex disease etiology, caused in part by large genetic heterogeneity among patients. Despite a clear genetic contribution to ALS, epigenomic molecular events such as DNA methylation (DNAm) may also play a role in ALS. DNAm differences between cases and could reflect both cause or consequences of disease. Here we applied the OmicS-data-based Complex trait Analysis (OSCA) software to new ALS (782 cases and 613 controls). OSCA implements mixed linear model approaches to account for unknown confounders by fitting a random effect of genome-wide methylation with variance-covariance structure estimated from the data and thus controls for the high false positive rate in standard linear models with little loss of power. We identified 10 significantly differentially methylated CpGs between ALS cases and controls and estimate the proportion of variance explained by all probes to be $\rho^2 = 16.1\%$. Finally, out-of-sample prediction with ALS derived BLUP solutions of the probes effects, gives significant predictive accuracy (AUC = 62%, $CI_{95\%}$= [0.59-0.64], p-value = $9.3x10^{-14}$). Given a reported genetic relationship between ALS and schizophrenia, we also assessed the predictive accuracy of ALS derived BLUP scores in an independent schizophrenia target sample and report a smaller but significant prediction (AUC = 54%, $CI_{95\%}$ = [0.51-0.57], p-value = $7.1x10^{-3}$).

M.F. Nabais: None.

P17.02C
Estrogen-mediated regulation of *FGF5* and its role for male-pattern baldness

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GWAS have identified >600 genetic risk loci for male-pattern baldness (MPB). However, for the majority of loci, the biological mechanisms through which the associated variants exert their functional effects remain elusive. Among them, the 4q21.21 risk locus located intronically in *FGF5*, a known inhibitor of human hair growth. Of note, premature termination of hair growth is a key pathophysiological sign of MPB. To gain a deeper understanding of the regulatory architecture at the *FGF5*-locus and its effect on MPB development a HaploRegv4.0 query was performed. The database research revealed that the lead SNP rs4690116 is located in an estrogen receptor 1 (ESR1) binding site. This is of particular interest, as estrogens have a reported role in hair growth control and act as opponents of androgens, the key hormonal mediators in MPB. To test if the risk allele may affect ESR1 binding affinity, which might eventually result in an insufficient inhibition of *FGF5* we designed a luciferase assay where we co-transfect (i) a luciferase vector containing the *FGF5* promoter and the 4q21.21-ESR1 binding site with either the risk or the alternate allele and (ii) an *ESR1*-expression vector. Estrogen-stimulation is used to induce ESR1 function. First results in HEK293T cells indicate an inhibition of the *FGF5* promoter under estrogen stimulation. Additional experiments are currently performed and the results will be presented at the conference. These data will elucidate the molecular mechanism at the *FGF5*-locus and will eventually contribute to a deeper understanding of MPB pathobiology.

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P17.03D
epigenome wide association studies in asthma: a systematic review

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Objective: Asthma is a common, chronic respiratory airway disease influenced by environmental factors and possibly their interaction with the human genome causing epigenetic changes. Epigenome wide association studies (EWAS) have mainly investigated DNA methylation and its association with disease or traits, exposure factors or gene expression. This systematic review aimed to identify all EWAS assessing differentially methylated sites associated with asthma in humans.

Design: Structured systematic literature search following PRISMA guidelines, Newcastle-Ottawa Scale for cohort studies was used for bias assessment.

Data Sources: We searched PubMed and Embase databases from 2005 through 2019.

Eligibility Criteria: EWAS studies testing association between differential methylation and asthma in humans.

Results: Overall, we identified sixteen EWAS studies complying with our search criteria. Twelve studies were conducted on children and ten were conducted on sample sizes less than 150 subjects. Four hundred and nineteen CpGs were reported in children studies after correction for multiple testing. In adult studies, thousands of differentially methylated sites were identified. Differential methylation in inflammatory related genes correlated with higher levels of gene expressions of inflammatory modulators in asthma. Differentially methylated genes associated with asthma included *SMAD3, SERPINC1, PROK1, IL13, RUNX3* and *TIGIT*. Fourty-one CpGs were replicated at least once in blood samples, and 28 CpGs were replicated in nasal samples.

Conclusion: Although many differentially methylated CpGs in genes known to be involved in asthma have been identified in EWAS to date, we conclude that further studies of larger sample sizes and analyses of differential methylation between different phenotypes are needed.

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P17.04A

Statistical finemapping and functional annotation of risk loci for Barrett's esophagus and esophageal adenocarcinoma

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Esophageal adenocarcinoma (EA) and its precancerous condition Barrett's esophagus (BE) are multifactorial diseases with rising prevalence rates in Western populations. A recent GWAS meta-analysis identified 14 risk loci, which are all located in non-coding genomic regions. Therefore, our understanding of the biological mechanisms underlying these associations is incomplete. The aim of this study was (i) to statistically finemap and (ii) to functionally annotate all known risk loci for BE/EA.

First, we calculated credible SNP sets for every risk locus. This resulted in sets of variants that most likely included true causal variants. The credible SNPs were cross-referenced with Roadmap chromatin states associated with enhancer activity from esophageal tissue. We functionally annotated variants located in enhancers and investigated nearby genes regarding their function. Based on this

information, the risk loci were prioritized for subsequent functional follow-up analyses.

As a first result, two credible SNPs at the 5p15 locus are located in an enhancer upstream *SLC9A3*, which encodes an epithelial brush-border Na-H-exchanger. Both SNPs, as well as the best-associated SNP at 5p15, are eQTLs for *SLC9A3*. Interestingly, upregulation of *SLC9A3* has been associated to gastroesophageal reflux disease - a major risk factor for BE/EA.

In order to unveil regulatory mechanisms that are involved in the development of BE/EA, we are now performing functional analyses. This includes 4C-sequencing and dual-luciferase assays in cells of all stages of BE/EA development. Altogether, our systematic approach of functional characterization of genetic risk factors will contribute to the elucidation of pathomechanisms involved in the development of BE/EA.

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P17.06C
Post-transcriptional mechanisms distinguish human and chimpanzee forebrain progenitors

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The human forebrain has increased in size and complexity since the split between the human and chimpanzee lineages and these changes are thought to underlie human-specific cognitive abilities. Interspecies divergence of regulatory elements as well as *trans*-acting factors are likely to contribute to human traits. Here we used bulk and single-cell RNA-seq in combination with in-depth proteomics to compare human and chimpanzee iPSC-derived forebrain progenitors. We found that interspecies differences in protein levels exceeded the differences in transcript levels. Lowly and moderately expressed proteins were found to contribute to most of the human-chimpanzee expression differences, while highly expressed proteins remained at similar levels. Our data indicate that post-transcriptional mechanisms and *trans*-acting elements play a crucial role in the evolution of the human forebrain.

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P17.07D
Identification of *CFTR cis*-regulatory variants

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Although, more than 2000 mutations have been discovered in *Cystic Fibrosis Transmembrane conductance Regulator* (*CFTR*) gene, some patients with cystic fibrosis or *CFTR*-related disorders (*CFTR*-RD) have incomplete genotypes or present extreme phenotypes. Development of chromatin conformation study techniques has identified several long-range regulatory elements as involved in this control expression. The objective of this project is to study the involvement of '*cis*-ruption', that is the dysfunction of a *cis*-regulatory element, in cystic fibrosis and CFTR-RD (*Congenital Bilateral Absence of Vas Deferens*, CBAVD). In a homogeneous group of 25 CBAVD patients carrying only one F508del mutation, 17 *cis*-regulatory regions of *CFTR* gene were sequenced. By comparing to European population, some variants display a frequency significantly different. In particularly, one variant located in *cis*-

regulatory region of intron 21 encompassing an important transcription factor binding site EP300, is 40 times more frequent in this group. Enhancer tests are realised to measure the effect of the intron 21 region on the activity of *CFTR* promoter in intestinal and airway cells. By combining the enhancer of intron 21 and the enhancer of intron 11 (strong enhancer described in intestinal cells), a strong cooperative effect is observed on the *CFTR* promoter activity in intestinal cells. These two enhancers have common transcription factor binding sites which some interact together (EP300/TCF12 and EP300/CEBPB). Enhancer tests with the insertion of the variant of interest in the combination of intron 11 and 21 enhancers are in progress to determine the impact of the variant on the CFTR promoter activity.

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P17.08A

NGS-panel for Chromatinopathies: implications in diagnosis and research

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Introduction: The regulation of the chromatin state by epigenetic mechanisms plays a central role in gene expression, development, function, and maintenance of cell identity. Defects in components of the epigenetic machinery lead to a highly heterogeneus group of syndromic conditions, we termed "Chromatinopathies" characterized by intellectual disability, growth abnormalities, and typical facial features.

Material and Methods: We designed a NGS-targeted panel containing 68 genes associated with chromatinopathies including Kabuki, Kleefstra, Coffin Lowry, Wiedemann Steiner, Rubinstein-Taybi, Floating Harbor, and Cornelia de Lange syndromes.

Results: Driven by clinical diagnosis, we analyzed 253 patients and found pathogenic variants in 60 (24%). The main sub-groups consist of 120 Kabuki syndrome patients, 33 (28%) of them carrying *KMT2D* or *KDM6A* pathogenic variants, Rubinstein-Taybi syndrome patients, with 12/47 (25%) subjects carrying *EP300* or *CREBBP* pathogenic variants and Floating Harbor syndrome patients, with 3/19 (16%) subjects carrying SRCAP pathogenic variants. Considering the clinical overlapping and shared molecular mechanisms among the Chromatinopathies, we extended the *in silico* variants analysis to all patients. 42/193 (22%) of patients showed pathogenic variants in genes not associated with the original clinical suspicion. A re-evaluation of the clinic signs in part confirmed the molecular data. Some examples include KS patients found mutated in *KMT2A*, *CTCF* and *ARID1B*; RSTS patients with frameshift and nonsense variants in *KMT2A*.

Conclusion: The study of chromatinopathies may offer a unique opportunity to learn about epigenetics in health and disease. This study highlights the need to analyze with NGS approaches these diseases that show molecular and clinical overlap.

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P17.09B

Expression study of candidate miRNAs and evaluation of their potential use as biomarkers of diabetic neuropathy

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Diabetic polyneuropathy (DPN) and Cardiovascular Autonomic Neuropathy (CAN) are common forms of diabetic neuropathy in patients with type 2 diabetes (T2D). There is an increasing evidence that microRNAs may be involved in many disorders as also in diabetes and its complications. Our aim was to evaluate the expression of candidate miRNAs in positive and negative patients for both forms of diabetic neuropathy. We recruited 50 T2D patients with neurological evaluation. We extracted RNA from peripheral blood mononuclear cells and quantified the expression of 6 miRNAs (miR-499a, miR-27a, miR-146a, miR-128a, miR-155, miR-21) by TaqMan assays. The expression in different groups was compared by ANOVA test. In

addition, we extracted DNA from the patients' whole blood, analyzed common polymorphisms of the MIRNA gene and evaluated the possible correlation between genetic variants and miRNA levels expression. Patients with DPN showed a higher expression of miR-128a compared to those DPN-negative (P=0.015). In contrast, miR-155 and miR-499 seem to be down-expressed in patients with DPN (P=0.04 and P=0.05, respectively). We observed a lower expression of miR-155 (P=0.05) even in patients with CAN respect to CAN-negative patients. Genotypic analysis showed that rs767649 polymorphism variant allele in the miR-155 promoter region is associated with a higher expression of this miRNA (P=0.003) compared to the wild-type allele. Our data suggest the involvement of miRNAs in the development of diabetic complications. If these preliminary results will be validated in more numerous cohorts, these miRNAs could be considered potential biomarkers for the development of diabetic neuropathy.

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P17.10C
DNA methylation regulates miR-146a expression in osteoarthritic synoviocytes by altering NF-kB binding affinity on promoter

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Introduction: Abnormal expression of miR-146a has been linked to osteoarthritis (OA) pathogenesis. We aimed to investigate the role of DNA methylation on miR-146a expression in OA synoviocytes.

Material and Methods: miR-146a expression levels were investigated in OA (n=16) and normal synoviocytes (n=9) using qRT-PCR. The methylation status of miR-146a promoter was analysed using quantitative methylation-specific PCR (qMSP) and bisulfite DNA sequencing assay. The effect of 5'-Aza-2-deoxycytidine (5-AzadC) and siRNA against NF-kB on miR-146a expression was investigated. In addition, NF-KB binding to *miR-146a* promoter was assessed by ChIP.

Results: miR-146a expression levels were significantly reduced in OA compared with normal synoviocytes. Downregulation of miR-146a was correlated with increased methylation of *miR-146a* promoter in OA synoviocytes and weaker binding affinity of NF-kB on this hypermethylated region in OA synoviocytes. Significant upregulation of miR-146a was observed in 5-AzadC-treated OA synoviocytes compared with untreated, which was associated with demethylation of miR-146a promoter. Inhibition of NF-kB reversed the effect of 5-AzadC on miR-146a expression in OA synoviocytes.

Conclusion: We demonstrated, for the first time to our knowledge, that DNA methylation regulates miR-146a expression in OA synoviocytes, by preventing NF-kB from binding to *miR-146a* promoter. These data provide strong evidence that epigenetic mechanisms could regulate the expression of miRNAs linked to OA pathogenesis. Fellowships: The study was co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Program «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers" (MIS-5001552) implemented by the State Scholarships Foundation (IKΥ).

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P17.11D
Enzymatic Methyl-Seq: Next Generation Methylomes

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DNA methylation is important for gene regulation. The ability to accurately identify 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) gives us greater insight into potential regulatory mechanisms. Bisulfite sequencing (BS) is traditionally used to detect methylated C's, however, BS does have its drawbacks. DNA is commonly damaged and degraded by the chemical bisulfite reaction resulting in libraries that demonstrate high GC-bias and are enriched for methylated regions. To overcome these limitations, we developed an enzymatic approach, NEBNext® Enzymatic Methyl-Seq (EM-Seq™), for methylation detection.

Illumina libraries were prepared using bisulfite and EM-Seq methods. Libraries generated with NA12878 DNA inputs ranging from 10 ng to 200 ng were sequenced using Illumina's NovaSeq 6000. EM-Seq libraries have longer inserts, lower duplication rates, a higher percentage of mapped reads and less GC-bias compared to bisulfite converted libraries. Global methylation levels are similar between EM-seq and whole genome bisulfite libraries

(WGBS) indicating overall detection of methylated C's is similar. However, CpG correlation plots demonstrated higher correlation coefficients indicating that EM-Seq libraries are more consistent than WGBS across replicates and input amount. GC-bias and dinucleotide distribution showed that EM-Seq has more even dinucleotide representation compared to the AT-rich representation observed for WGBS. EM-seq's more even coverage allows for a higher percentage of CpG's to be assessed leading to more consistent evaluation of methylation across key genomic features (TSS, CpG island, etc.). EM-seq is more robust than WGBS, works over a wide range of DNA input amounts, has superior sequencing metrics, and detects more CpG's.

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P17.12A
DNA methylation signatures as potential novel prognostic biomarkers in sub-types of Non-Small Cell Lung Cancer (NSCLC)

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Introduction: NSCLC constitutes the most common cancer-related cause of death. Adenocarcinoma (AC) and squamous cell carcinoma (SCC) are characterized by unique molecular features. Recent studies have revealed that cancer-related changes in DNA methylation are not random, and subsets of genes are selectively affected. The aim of the research was to characterize methylome profiles of AC and SCC tumors, and to develop novel prognostic biomarkers specific for both sub-types.

Materials and Methods: For large-scale methylome profiling, reduced representation bisulfite sequencing (RRBS) in 123 patients with NSCLC was carried out. The paired analyses of lung tumor and adjacent normal tissue of patients grouped into several comparison sets was performed. Then, using binary segmentation algorithm, differentially methylated regions (DMRs) were identified.

Results: Hundreds of DMRs were identified in NSCLC; vast majority of them exhibited hypomethylation in patients with relapse or who died. In tumors of SCC patients with relapse, several DMRs were detected within *GATA2* gene, playing a role in an aggressiveness of lung cancer and especially important in patients with *KRAS* mutations. Interestingly, a distinctive methylation levels were observed for *RUVBL1*-related DMRs in SCC and AC patients with relapse. Hypomethylation of *PRKCA*, which exhibits suppressor activity in lung cancers, was identified in AC patients who eventually did not survive.

Conclusion: Obtained results allow for better understanding of specific epigenetic pathways dysregulated in different sub-types of NSCLC. We have provided a catalog of methylation-related biomarkers predictive in terms of tumor relapse and survival.

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P17.13B
An integrated chromatin accessibility and transcriptome landscape of human pre- and post-implantation embryos

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Introduction: The human pre- and post-implantation development is a delicately balanced and orchestrated process that involves extensive changes in chromatin

structure and transcriptional activity. However, a genome-wide survey of chromatin structure and its association with molecular regulation in this process have been impeded by the scarcity of the required materials.

Materials and Methods: For pre-implantation development study, we adopted LiCAT-seq, a technique that allows simultaneous profiling of chromatin accessibility and gene expression with ultra-low input of cells, cell stages including oocytes, 1-cell, 2-cell, 4-cell, 8-cell, morula and blastocyst. For post-implantation development study, we generated single-cell RNA and single-cell ATAC-seq data from E6, E7, E8, E9, E10, E12, E13.5 cells using an *in vitro* culture system. Here, combined with transcriptome and epigenome maps of human pre- and post-implantation *in vitro* culture system (before E14) allowed us to reveal the underlying regulatory mechanism of cell fate decision during this complex developmental time.

Results: Integrative analysis between the two omics layers revealed a strong association between the establishment of accessible chromatin and the related genes up-regulated during embryonic genome activation (EGA) and epiblast development. Furthermore, combined analysis of transcription factor accessibility identified putative novel transcription factors in regulating EGA and cell fate decision. In addition, we identified massively expressed endogenous retrovirus (ERVs) during EGA and epiblast disc.

Conclusions: we mapped the chromatin accessibility and transcriptome profiles for human pre- and post-implantation embryos. Our results thus offer new mechanistic insights into the molecular events inherent to human pre- and post-implantation embryo development.

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P17.14C
CRUP: A comprehensive framework to predict condition-specific regulatory units

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Gene expression is to a large degree regulated by distal genomic elements referred to as enhancers, which recruit a combination of different factors to activate transcription from a targeted core promoter. The activity state of enhancers may change dynamically across distinguishable conditions, for instance across varying time-points, cell lines or disease states. However, it remains a challenge to identify these regulatory elements in a cell-type or even disease-state dependent manner. Thus, rather than comparing separated epigenetic signature tracks we propose an approach to computationally map and compare enhancers across different samples and conditions. Additionally, to get a glimpse of the underlying causative regulatory mechanism, these dynamic enhancer elements need to be further associated with promoter activity across the same conditions.

Here we present the three-step framework CRUP (Condition-specific Regulatory Units Prediction) to collapse different layers of epigenetic information into a single list of regulatory units consisting of dynamically changing enhancers and target genes. The workflow includes a pre-trained enhancer classifier that can be applied across different cell lines and species, solely based on histone modification ChIP-seq data. Enhancers are subsequently assigned to different conditions and correlated with gene expression to derive regulatory units within the same topologically associated domain.

We thoroughly test and then apply CRUP to murine embryonic stem cells to capture dynamic enhancer regions that are associated with retinoic acid signaling. Finally, we identify trait-associated regulatory elements in a mouse study of rheumatoid arthritis and identify enhancer-gene pairs comprising known disease genes as well as new candidate genes.

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P17.15D
Deletion of *FOXG1* transcriptional enhancers is associated with Rett-like syndrome

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Gene regulatory elements such as enhancers dynamically regulate gene expression in a tissue-specific manner. However, the transcriptional regulatory elements during human inhibitory interneuron differentiation and their role in neurodevelopmental disorders are unknown. Here, we generated gene regulatory element maps of human inhibitory-like interneurons derived from embryonic stem cells (H9-ESC), permitting large-scale annotation of previously uncharacterized regulatory elements relevant to inhibitory interneuron differentiation. Our analyses identify neuronal progenitor enhancers that likely regulate the expression of transcription factors that are essential for interneuron differentiation. One of these transcription factors is FOXG1 that is specifically expressed in interneuron progenitors. Haploinsufficiency of FOXG1, is associated with Rett-like syndrome. Using *in vivo* enhancer assay, we identified eight transcriptional enhancers in the *FOXG1* locus with activity patterns that resembled *FOXG1* expression. Using CRISPR/Cas9 genome editing, we deleted two *FOXG1* enhancers which reduced FOXG1 expression in human U251 cells and altered cell proliferation. Furthermore, a microdeletion proximal to FOXG1 encompassing these neuronal FOXG1 enhancers was found in patient with Rett-like syndrome, supporting the role of *FOXG1* enhancers in this syndrome. Our study provides a framework for understanding the impact of non-coding regulatory elements during inhibitory interneuron differentiation, and highlights novel mechanisms underlying neurodevelopmental disorders.

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P17.16A

Barrett's esophagus - functional annotation of genetic associations using tissue-specific gene expression and the FUMA platform

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Barrett's esophagus (BE) is a premalignant lesion that predisposes to esophageal adenocarcinoma (EA). Both diseases are multifactorial, and GWAS meta-analyses have identified 14 risk loci so far. All risk variants are located in non-coding genomic regions and therefore our understanding of biological mechanisms underlying these associations is incomplete. Here, we aimed at (i) characterizing BE/EA genetic associations using eQTL data from two relevant tissues, and (ii) investigating the biological function of eQTL genes. For this, we generated two eQTL datasets from biopsies derived from healthy gastric cardia tissue (n=148), and from esophageal metaplasia tissue (n=83). The eQTL analysis revealed 145,011 significant cis-eQTLs in cardia and 50,517 significant cis-eQTLs in metaplasia tissue. We cross-referenced the eQTL data with genetic association data of BE/EA. Here, we identified cis-eQTL effects for genome-wide significantly associated BE/EA risk variants regulating the expression of *C2orf43/LDAH* (2p24) in cardia tissue. In metaplasia, only suggestively associated variants ($P<5\times10^{-04}$) represented cis-eQTLs, with regulating effects on *SMPD2* (6q21) and *NADSYN1* (11q13). Next, we performed a functional enrichment analysis with eQTL genes present in cardia tissue only (n=2,557), metaplasia tissue only (n=1,028), and both tissues (n=585). We observed an enrichment of metaplasia eQTL genes in immunological pathways, whereas cardia eQTL genes were enriched in metabolic processes. eQTL genes expressed in both tissues were enriched in pathways involved in degradation of extracellular matrix. In summary, our results point towards specific biological pathways that play a role in healthy and

disease states of tissues that are involved in the pathophysiology of BE.

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P17.17B
Droplet digital PCR-mediated evaluation of *ESR1* promoter methylation in cell-free DNA

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Introduction: The use of circulating cell-free DNA (ccfDNA) is gaining momentum offering the possibility to assess disease progression and therapeutic response in a non-invasive manner. Tumor expression of estrogen receptor is an important marker of prognosis and is predictive of response to endocrine therapy in luminal breast cancer (BC). Indeed, promoter hypermethylation could lead to a change in *ESR1* gene expression profile, which interferers with endocrine therapy, leading to resistance.

Materials and Methods: ccfDNA was isolated from plasma samples from 11 metastatic breast cancer patients and 8 healthy volunteers. For absolute quantification of *ESR1* promoter methylation levels, bisulfite-converted ccfDNA was used and ddPCR was performed with two probes, one specific for methylated DNA and one specific for un-methylated DNA.

Results: *ESR1* promoter methylation status was tested on ccfDNA samples using ddPCR. To avoid any effect of incomplete bisulfite conversion on accuracy of methylation detection, primers pairs were designed to target only converted cytosine residues. *ESR1* promoter resulted unmethylated in all healthy donors' ccfDNA samples. A variable degree of methylation was assessed between patients' samples.

Conclusions: Droplet digital PCR methodology is an endpoint PCR method that does not require standard curves for absolute quantification and can be easily optimized to assess low-amount and fragmentated samples such as ccfDNA. Our data demonstrated that ddPCR can be a valuable tool for the precise and accurate detection of *ESR1* promoter methylation on clinical samples, allowing the assessment of small percentage of methylated DNA, with a limit of detection of 2% methylation.

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P17.18C
New insights on the role of noncoding RNAs in the pathology of Fragile X-associated disorders

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Introduction: Currently, based on the size of the CGG repeat in the *FMR1* gene and the methylation status of its promoter region, the variation in symptom severity in Fragile X-associated disorders cannot be explained. In this study, our hypothesis assumed that non-coding RNAs, such as miRNAs and *FMR1* antisense transcripts, are involved in the pathology of Fragile X-associated disorders.

Materials and Methods: Immortalized B-lymphocyte cell lines from patients with different *FMR1* gene alleles were used. The expression of genes and miRNAs were analyzed by SYBR and TaqMan PCR, respectively. MicroRNAs were selected using the miRBase, microRNA and TargetScan databases.

Results: Using the databases, a number of miRNAs that bind to the 3'-UTR of the *FMR1* gene were selected. It was shown that the expression of some miRNAs in groups with different *FMR1* gene activities changes relative to control cell lines. Next, it was shown that the expression of the antisense transcript, *ASFMR1*, which contains a GCC repeat and is a potential source of miRNA, as well as the general expression of all isoforms, also changes relative to controls. Based on the results, a new model is proposed for the participation of noncoding RNAs in Fragile X-associated pathologies. The reported study was funded by Russian Science Foundation project 18-15-00099.

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P17.20A
Dissecting the gene regulatory landscape of human adipose tissue

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Introduction: Subcutaneous (S) and visceral (V) fat share extensive biology, but a fraction of molecular processes remains particular to each tissue. Given the contribution of adipose tissue to disease risk, we aimed to uncover biological signatures characteristic of each tissue, and to explore fine-scale gene regulation.

Materials and Methods: Blood and biopsies of S and V fat were collected for 106 Greek individuals (GM study), spanning the BMI range. We explored gene expression (RNA-Seq) and chromatin accessibility (ATAC-Seq) in both tissues, and applied QTLtools software to identify regulatory variants (eQTLs). We explored replication of findings in GTEX (pi1, bootstrapping) and across tissues (pi1, RTC, linear models) to uncover associations characteristic of the GM sample and of each tissue respectively.

Results: Gene expression and chromatin accessibility differences across S and V fat reveal processes linked to development, cell signaling, and immune and nervous systems. Notably, we detected significant downregulation of metabolic processes in S fat from obese individuals. We report that ~90% of associations replicate in GTEX, but define a subset of GM-detected findings likely driven by environmental effects. Although ~95% of eQTLs are common across tissues, we uncover gene regulatory differences at the fine-scale, and highlight subtle differences in gene regulation across tissues. We also report that ~30% of eQTLs explain disease association signals.

Conclusions: Our findings contribute to a better comprehension of fat depot-specific biology providing insights into biological mechanisms underlying adipose-related disease pathogenesis. Funding sources: Marie Curie IEF, EMBO, Greek Ministry of Education "Thales" grant, Stavros Niarchos Foundation.

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P17.21B
A complete strategy for characterizing on- and off-target CRISPR/Cas9 editing events via target enrichment and high-resolution NGS analysis

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Genome editing with the CRISPR/Cas9 system is moving towards therapeutic applications, which drives an increased need for in-depth characterization of both on- and off-target genome editing events. Here we present a workflow and useful guidelines for prediction, validation, quantification, and analysis of on- and off-target editing events. First, we provide comparison of several commonly-used off-target prediction tools for *in silico* selection of target sites throughout the genome. Then, using previously-published, unbiased methods for experimental off-target validation, we are able to compare these algorithm-based selections to bona fide sites exhibiting editing mediated by more than twelve guide RNAs. The unbiased detection methods are carried out with experimental and bioinformatics advancements to improve the efficiency of empirical off-target validation. A multiplexed, amplicon-based enrichment method (rhAmpSeq^{TM}) for next-generation sequencing is then employed for strict quantification of editing events at validated as well as predicted off-target sites. The rhAmpSeq technology enables interrogation of >1000 genomic loci in a single reaction using RNase H2-cleavable primers which facilitate almost complete suppression of non-target location and primer dimer amplification. Moreover, amplicon coverage is uniform and reproducible, removing the need for primer re-balancing. The relationship between coverage depth and statistical confidence in quantification of editing events was characterized. Finally, we developed and rigorously validated a data analysis package to confidently detect Cas9 edits via synthetic reads that contain diverse editing events and varied genomic target complexities.

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P17.23D
Functional characterization of a lncRNA in the autoimmune disease-associated*RGS1* region

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Genome-wide association studies (GWAS) have helped in the identification of SNPs associated to different disorders. However, many of those variants are intergenic and some are located on functionally uncharacterized long non-coding RNAs (lncRNAs), several of which have been described to play crucial roles in many inflammatory disorders. rs2816316 (chr1:192567683) is located near the gene *RGS1* (regulator of G protein signalling 1) that has been related to the immune response. The variant has been associated with Celiac Disease (CD) and Multiple Sclerosis (MS), while *RGS1* has been related to CD, MS and Type 1 Diabetes (T1D). The aim of this study is to functionally characterize the region under this associated SNP in order to explain its implication in autoimmune disorders. The expression of *RGS1* is altered in the immune cells of rs2816316-associated diseases. According to GTEX rs2816316 is an eQTL for *RGS1* in gastroesophageal and brain tissues. The UCSC Genome Browser features a functionally uncharacterized lncRNA that contains the SNP and is close to *RGS1*. We have observed that this lncRNA is expressed both in immune and epithelial cells and localizes to the nucleus, suggesting a transcription regulatory role. Additionally, CRISPR-Cas9 edition of cells showed that the lncRNA and *RGS1* present opposite expression trends. Stimulation of Jurkat T cells with PMA and ionomicyn provoked a time-shifted activation of *RGS1* and the lncRNA. Our results suggest that the region around rs2816316 is involved in *RGS1* regulation, probably through a nearby nuclear lncRNA. Funding: PI16/00258 (JRB), EJ-2017111082 and ACM (ACR) and PRE_2017_1_0306(AOG).

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P17.24A
Comparative effects of Dasatinib and Ponatinib on lncRNA expression in Chronic Myeloid Leukemia

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Chronic myeloid leukemia-(CML) is characterized by cells carrying BCR-ABL1, so tyrosine kinase inhibitors-(TKIs) are ideal for selective treatment. Although many studies are conducted related to the mechanisms of action for TKIs like Dasatinib and Ponatinib, their effects on long non-coding RNAs-(lncRNAs) expression profiles which are associated with malignancies with their oncogenic/tumor suppressor properties are largely unknown. In this study, we aimed to define lncRNAs involved in the treatment of CML with Dasatinib and Ponatinib. Cytotoxicity, apoptosis and lncRNA expression profiles were evaluated by WST-8 assay, APO-DIRECT in flow-cytometry and qRT-PCR, respectively. Ponatinib exhibited higher cytotoxic and apoptotic activity than Dasatinib in K562-cells. Apoptosis-regulating lncRNAs HULC, HOTAIR, HOXA3AS and ANRIL that suppresses the DNA-damage response genes were down-regulated, while transcription-controller 7SK was up-regulated with both TKIs. Among lncRNAs associated with the pathways that are activated by BCR-ABL1; BCMS and PTENP1 were regulated with both TKIs, while GAS5 was only regulated with Ponatinib. In addition to the up-regulation of WT1-AS that suppresses WT1 oncogene by binding to its promoter, a prominent increase in Zeb2NAT and devastating decreases in PSF-inhibiting-RNA, BC017743, SCA8, and HOTTIP were specific to Ponatinib treatment. NCRMS, HOTAIRM1, Gomafu alterations were associated with Dasatinib-specific response. Our study reveals that numerous lncRNAs are involved in anti-leukemic activities of Dasatinib and Ponatinib treatment. These results will contribute to understanding signalization occurring in CML cells after standard treatment.

Expression profile of lncRNAs in K562 cell line after treatment with Dasatinib or Ponatinib

lncRNAs similarly regulated with Dasatinib and Ponatinib			lncRNAs regulated with Dasatinib		lncRNAs regulated with Ponatinib	
lncRNA Symbols	Fold Change with Dasatinib (Log2 Transformed)	Fold Change with Ponatinib (Log2 Transformed)	lncRNA Symbols	Fold Change with Dasatinib (Log2 Transformed)	lncRNA Symbols	Fold Change with Ponatinib (Log2 Transformed)
Zeb2NAT	2.13	15.91	***TEA ncRNAs***	18.63	***WT1-AS***	5.27
PTENP1	3.08	2.15	***H19***	16.44	***HAR1B***	4.88
7SK	3.11	2.14	***NCRMS (RMST)***	16.20	***21A***	3.55
PCAT-43	-13.29	-2.46	***DLG2AS***	12.42	***LUST***	2.60
BCMS (DLEU1)	-2.29	-2.55	***PRINS***	6.69	***BACE1AS***	2.26

LincRNA-VLDLR	-4.56	-2.61	*DHFR upstream transcripts*	2.30	*GAS5*	2.21
HOTAIR	-3.83	-2.77	*ST7OT1*	-2.00	*NDM29*	2.06
HULC	-2.88	-2.92	*HAR1B*	-2.09	*TEA ncRNAs*	-2.11
MER11C	-4.98	-5.53	*Sox2OT*	-2.52	*aHIF*	-2.58
LincRNA-SFMBT2	-4.19	-5.76	*ST7OT2*	-2.53	*BC200*	-2.73
HOXA3AS BE873347	-4.34	-6.76	*LUST*	-2.60	*IPW*	-2.97
PCAT-29	-5.12	-7.16	*BIC*	-2.68	*TU_0017629*	-3.35
ANRIL	-12.64	-11.46	*NEAT1*	-2.83	*DLG2AS*	-4.71
PCAT-1	-9.04	-12.36	*anti-NOS2A*	-3.21	*ST7OT4*	-6.13
PCAT-14	-7.56	-12.40	*PR-AT2*	-3.35	*PRINS*	-6.77
ST7OT3	-3.05	-14.47	*L1PA16*	-3.37	*SCA8*	-9.87
PCAT-32	-8.90	-16.16	*LIT*	-3.39	*HOTTIP*	-11.17
PSF inhibiting RNA	-2.25	-17.29	*HOXA3AS BI823151*	-4.11	*AAA1*	-14.29
			NDM29	-5.23	*BC017743*	-16.53
			HOTAIRM1	-6.01		
			Gomafu (MIAT)	-9.12		
			Air	-18.14		

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P17.25B
Analysis of miRNA expression in non-small cell lung cancer

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Introduction: MicroRNAs(MiRNAs) can help understanding the carcinogenesis of lung cancer and serve as potential diagnostic biomarkers for differentiating adenocarcinoma (ADC) and squamous cell lung carcinoma(SCC).The aim of the present study is to analyse and compare the expression patterns of miRNAs in ADC and SCC samples

Materials and Methods: Fresh frozen tissue samples from 24 non-small cell lung cancer(NSCLC) patients (12ADC,12SCC) and adjacent normal tissues were examined. The expression of miRNAs was evaluated by SurePrint human miRNA microarrays (Agilent Technologies). The normalization of data and the statistical analysis were performed by GeneSpring GXsoftware.

Results: We assessed the expression levels of 2549 human mature miRNAs and found 13 to be significantly differentially expressed (fold change (FC)>2.0 and FDR<0.005) between the ADC and adjacent normal tissues. Hsa-miR-210-3p, hsa-miR-21-3p and hsa-miR-130b-3p were upregulated. One hundred twenty eight miRNAs were significantly differentially expressed (FC>2.0 and FDR<0.001) between the SCC and adjacent normal tissues of which 56 were upregulated and 72 downregulated.Hsa-miR-30a-5p(FC=-6.48) and hsa-miR-195-3p(FC=-21.07) were significantly downregulated in ADC,whilehsa-miR-30a-3p(FC=-210.94) and hsa-miR-195-5p(FC=-154.26)in SCC.In SCC we also identified 6 aberrantly expressed miR-5p/-3p pairs that potentially modulate the gene expression of proteins:hsa-miR-140-3p/hsa-miR-140-5p,hsa-miR-143-3p/hsa-miR-143-5p,hsa-miR-145-3p/hsa-miR-145-5p,hsa-miR-29c-3p/hsa-miR-29c-5p,hsa-miR-30b-3p/hsa-miR-30b-5p and hsa-miR-532-3p/hsa-miR-532-5p.

Conclusions: The expression patterns of miRNAs and their target genes revealed both common and subtype specific signal pathways for ADC and SCC. Our results were in agreement with previous suggestions that miR-5p/-3ppairs coregulated protein interaction networks critical to lung cancer development. However further analysis of enlarged sample and validation of the microarray results by qRT-PCR is necessary to ascertain their diagnostic potential in NSCLC.

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P17.26C
Peripheral blood DNA methylation as prognostic tool in Malignant Pleural Mesothelioma

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Introduction: Malignant pleural mesothelioma (MPM) is a rare and aggressive neoplasm, with limited systemic therapeutic options and median survival time of approximately 12 months. The aim of this study was to evaluate the clinical value of DNA methylation (DNAm) in predicting overall survival (OS) as compared to the lymphocyte-to-monocyte ratio (LMR), which is the most used inflammation-based prognostic score in MPM.

Materials and Methods: We investigated a cohort of 163 incident cases of MPM diagnosed between 2000 and 2010 in the municipalities of Turin, and Casale Monferrato (Piedmont region, Italy), an area with an exceptionally high incidence of mesothelioma caused by asbestos occupational exposure and contamination in the general environment from the asbestos-cement Eternit plant that was operational until 1986. Genome-wide methylation array (HumanMethylation450 Beadchip) to identify novel blood DNAm markers related to overall survival in MPM was used.

Results: Kaplan-Meier survival curves highlighted methylation levels at a single-CpG in a gene on 6p21.31 (DNAm cut-off = 0.45, HR = 2.14, Median Survival = 243 vs 534, days; $P = 2.4 \times 10^{-05}$) as related to OS.

Conclusions: Our study is the first to demonstrate that a single-CpG DNAm in a gene on 6p21.31 is an independent marker of prognosis in patients with MPM and performs better than other inflammation-based scores as prognostic factor. DNAm evaluation will enable clinicians to better predict clinically meaningful outcomes such as response to systemic treatment and to select patients who are most likely to benefit from intensive therapy.

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P17.27D

Methylation alterations in developmental and differentiation genes drive resistance to immunotherapy in a melanoma model

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Background: Therapy resistance limits the efficacy of immunotherapy in melanoma patients. We have reported that phenotypic tumor cell plasticity in a pro inflammatory microenvironment drives therapy resistance to adoptive T cell therapy (ACT). We suggest that pro-inflammatory responses induce a dedifferentiated phenotype and therapy resistance by changing the epigenetic landscape. Here, we performed an integrative analysis of exome, methylome and transcriptome profiles of primary and ACT therapy resistant cell lines in transgenic mouse model of melanoma.

Methods: To detect if ACT selects for common mutations in resistant cell lines, we performed whole exome sequencing (WES) in primary and therapy resistant cell lines. We used Methylated DNA immunoprecipitation coupled with next generation sequencing (MeDIP- Seq) to track the distribution of methylation alterations in target genomes. Genome wide expression data are generated using RNA sequencing.

Results: We did not find a driver alteration in somatic mutation patterns. Differential methylation (DM) analysis for all samples shows profound clustering for primary cell lines against therapy resistant cell lines. Differential expression (DE) analysis results are consistent with profound DM clustering in our cell lines. Gene set enrichment and biological pathways analyses for significant DE and DM genes show the highest enrichment scores for developmental pathways and differentiation proteins.

Outlook: Our results supports the hypothesis that significant changes in methylation marks drive melanoma cell plasticity and immunotherapy resistance in our experimental model. Further functional studies would shed light on the correlation of “inflammatory tumor microenvironment” and epigenetically driven “resistant phenotypes” in melanoma.

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P17.28A

Identification of blood *cis* expression quantitative trait methylations (eQTMs) in children

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The identification of expression quantitative trait methylations (eQTMs), defined as correlations between gene expression and DNA methylation levels, might help the biological interpretation of epigenome-wide association studies (EWAS). Here, we aimed to identify *cis*-eQTMs in child blood using data from 832 children of the Human Early Life Exposome (HELIX) project.

Blood DNA methylation and gene expression were assessed with the 450K and the HTA v2 arrays, respectively. The relationship between methylation levels and expression of nearby genes (transcription start site within 1 Mb window from CpG) was tested by fitting 13,615,882 linear regressions adjusting for sex, age, cohort and cell type composition.

After Bonferroni correction, we found 15,403 *cis*-eQTMs, 9,477 (61.5%) of them showing an inverse association. They included 8,907 unique CpGs and 3,790 unique genes, and mean distance between them was 240 kb. *Cis*-eQTMs were enriched for distant promoters, N and S CpG island shores, and transcriptional and enhancer blood chromatin states. Genome-wide genotypic data is available for these same children and it will be used, in future steps, to investigate to which extend *cis*-eQTMs are determined by genetic variants.

This catalogue of *cis*-eQTMs will be useful for understanding DNA methylation effects on blood gene expression during childhood.

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P17.29B

Methylation anticipation extends to the outside of ICR1 in familial Beckwith-Wiedemann syndrome patients with ICR1 mutation

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Introduction: Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder. Gain of methylation at ICR1 (ICR1-GOM), one of the causative alterations for BWS, is found in 5% of patients, and 20% of ICR1-GOM patients have mutations of OCT-binding motif within ICR1. Methylation anticipation of ICR1 upon maternal transmission of the mutation in familial BWS patients was previously reported. However, the extent of anticipation within *IGF2*/*H19* imprinting domain is still unclear.

Materials and Methods: Members of BWS family with an OCT mutation were subjected to this study. Methylation statuses of 10 differentially methylated sites, such as *IGF2*-DMR0, *IGF2*-DMR2, CTS1-7, and H19-promoter, were analyzed by quantitative bisulfite-pyrosequencing.

Results: A patient, mother, and aunt with the mutation showed ICR1-GOM, however, grandmother with the mutation and other normal family members did not. CTS2, the second nearest CTS to the mutation, was the most highly methylated site among all sites. Not only sites within ICR1 (CTS 1-6) but also sites outside ICR1 (*IGF2*-DMRs, CTS7, and H19-promoter) were more methylated in the patient than in mother and aunt, indicating the methylation anticipation. Furthermore, the extent of anticipation was greater outside ICR1 than inside ICR1. In addition, *IGF2*-DMR0 was hypomethylated in normal young adults than in normal children, and it was maintained to middle-aged adults.

Conclusions: Methylation anticipation of ICR1 might be due to failure of maternal methylation erasure during female gametogenesis. The extent of anticipation was greater at the sites far from the mutation. Hypomethylation of *IGF2*-DMR0 normally occurred in people underage.

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P17.30C
Genome-wide DNA methylation analysis in two different types of pituitary tumors

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Pituitary neuroendocrine tumor (PitNETs), are benign tumors, distinguished in "functioning" (secreting hormones, causing a hormonal hyperfunction syndrome) such as Growth hormone (GH) - secreting and "non-functioning pituitary adenoma" NFPA. Usually, PitNETs are associated with many health and mortality complications when not adequately treated. Transphenoidal surgery is considered the treatment choice of PitNETs, followed by medical therapy. Sometimes the treatment is limited to selected cases, whereby, somatostatin analogs (SSA) are used in GH-PitNET. However, 10-30% of patients are not responder to SSA, suggesting that other molecular mechanisms may be essential for the response to drug treatment. Therefore, the identification of these mechanisms, allows to classify into subgroups PitNET patients (responders vs. non-responders). Epigenetic mechanisms can play a dynamic role in complex diseases such pituitary cancer. These mechanisms, including DNA methylation, influence the regulation of genome and the cell function. Our goal is to identify the differential methylation between GH-secreting and NFPA tissues.

Methods: We analyze DNA methylation data across the genome using (a) traditional coupled t-tests to identify significantly differentiated methylated loci ($P \leq 1 \times 10\text{-}7$ adjusted with Bonferroni) and (b) new combinatorial algorithms to identify loci that distinguish between types of fabrics.

Results: We obtained 178 target regions that were differentially methylated (corrected P value ≤0.05). Furthermore, only two regions were significantly hypomethylated in GH compared to NFPA. We have also elucidated gene-centric annotations (with ANNOVAR software) to obtain distances from the closest genes and other genomic information relative to the priority regions to fuse these 3 different pituitary diseases.

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P17.33B
Mitoepigenetics and neurodegenerative diseases

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Introduction: In recent years growing evidence on a potential role of altered mitochondrial epigenetic mechanisms (mitoepigenetics) in several diseases have emerged but until now little attention has been given to neurodegenerative diseases (NDs). Recently, we reported that methylation levels of the mitochondrial displacement loop (D-loop) region are impaired in peripheral blood of late-onset Alzheimer's disease (AD) patients, and in amyotrophic lateral sclerosis (ALS) patients with mutations in the *SOD1* gene. The major aim of this research is to further improve our knowledge on the potential role of mitoepigenetic imbalance in neurodegeneration.

Materials and Methods: Blood samples have been collected from a new cohort of 80 AD and 50 ALS and in 80 Parkinson's disease (PD) patients as well as from matched controls. DNA methylation analyses have been performed by means of MS-HRM and pyrosequencing and mtDNA copy number by means of quantitative PCR.

Results: DNA methylation analysis showed that D-loop methylation is able to discriminate disease patients and control subjects, and that it is also sensitive to the stage of the disease. Moreover, D-loop methylation levels have been found to be inversely correlated with mtDNA copy number and to be highly related to age and gender of the individuals enrolled.

Conclusions: Results presented in the current study suggest a potential involvement of mitoepigenetics imbalance in NDs detectable in peripheral blood. Moreover, current results suggest that mtDNA methylation could be sensitive to different disease stages, thus adding a new layer of interest in the search of peripheral mitoepigenetic biomarkers for neurodegeneration.

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P17.34C

Polymorphisms in folate metabolic genes contribute to *MTHFR* promoter methylation levels

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Introduction: The *MTHFR* gene codes for methylenetetrahydrofolate reductase, one of the major enzimes involved in folate metabolism. Impaired *MTHFR* methylation levels are increasingly recognized to contribute to several human pathological conditions, including male infertility, miscarriage, congenital heart defects, stressful events, cancer, and diabetic complications among others. Previous studies have clearly shown a correlation between circulating folate or homocysteine levels and *MTHFR* promoter methylation and gene expression, but little is still known concerning the contribution of polymorphisms in folate metabolic genes to *MTHFR* promoter methylation levels.

Materials and Methods: Two published datasets containing data on *MTHFR* promoter methylation and of major polymorphisms in folate metabolic genes have been investigated searching for correlation between those polymorphisms and *MTHFR* promoter methylation levels, for a total of 300 subjects. Both datasets had been previously generated in our laboratory in the frame of a dementia case-control study and of a screening for maternal risk factors for Down syndrome, respectively.

Results: In both datasets under investigation, as well as in the merged samples, we observed a correlation between *MTHFR* promoter methylation levels and both *TYMS* 1494del6 ($P = 0.007$) and *DNMT3B* -149C>T ($P = 0.004$) polymorphisms.

Conclusions: Present results reveal that certain polymorphisms of folate metabolic genes contribute to *MTHFR* methylation, suggesting that the expression levels of the *MTHFR* gene are tightly regulated by both dietary and genetic factors.

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P17.35D

NSD1 Mutation in Head and Neck Squamous Cell Carcinoma and its effects on histone modification and DNA methylation

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Introduction: DNA alterations and the cell-signaling pathways involved in HPV(-) HNSCC have been studied extensively, but we are far from understanding the underlying mechanisms of tumorigenesis. Recently, a subgroup of HPV(-) HNSCC has been characterized by mutations in the Nuclear Receptor-binding SET Domain protein 1 (NSD1), suggesting a possible epigenetic etiology of those tumors.

Method: We used two HNSCC cell lines carrying NSD1 truncating mutations (SKN-3 and SCC-4) and compared them with two NSD1-wildtype cell lines (Detroit562 and Cal27). In the next step, I produced isogenic cell lines by knocking out NSD1 gene in three NSD1-wild type HNSCC cell lines (FaDu, Detroit562, and Cal27) using CRISPR-Cas9 method to see the same result was observed or not. Finally, I chose 2 samples from my Cal27 NSD1-knockouts and knocked out NDS2using the same method, but with differently designed primers.

Results: Firstly, disruption of NSD1— in HNSCC samples where NSD1 is naturally disrupted— leads to a global reduction in the H3K36me2 histone modification at the intergenic regions, which is accompanied by a reduction in DNA methylation. Secondly, it was shown that the decrease of H3K36me2 mark in intergenic regions is correlated with increased H3K27me3 in those same regions.

Conclusion: So far, we demonstrated that a drastic and global decrease in DNA methylation at intergenic regions is directly associated with the lack of H3K36me2 mark in the same regions. This study will more specifically investigate the role of NSD1 and H3K36me2 mark and will uncover its correlation with H3K27me3 in HNSCC.

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P17.36A

Identification of aberrantly expressed long non-coding RNAs in osteoblastic cells from osteoporotic patients

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Introduction: Osteoporosis (OP) is a multifactorial disease influenced by both genetic and environmental factors. The major cause of the bone homeostasis alteration is inflammation. Epigenetic factors represent a link between individual genetic susceptibility and environmental influences associated with osteoporosis risk. In particular, long non-coding RNAs (lncRNAs), have a crucial role in regulating many important biological processes in bone, including inflammation.

Materials and Methods: We designed our study to identify lncRNAs misregulated in bone cells from OP patients with the aim to predict possible RNA and/or protein targets implicated in this multifactorial disease. Total RNA was extracted from osteoblast primary cultures derived by OP (n=5), and CTRs (n=5) individuals. Gene expression has been focused on 84 lncRNAs, validated or predicted, to regulate the expression of inflammatory genes and miRNAs. *In silico* analysis using validated bioinformatics tools has been utilized to predict the interaction of lncRNAs with miRNAs, mRNAs and proteins targets.

Results: Seven lncRNAs were significantly down-regulated in OP patients compared to controls: GRM5-AS1; CEP83-AS1; CTC-487M23.5; GAS5; RP11-84C13.1; NCBP2-AS2 and SDCBP2-AS1. Bioinformatics analyses identified two lncRNAs prediction targets that are implicated in bone homeostasis and in OP: CTC-487M23.5 that interacts with HDAC2 mRNA (a key positive regulator of bone resorption) and GAS5 a regulator of miR-21-5p, which itself interact with PTX3 mRNA (a novel regulator of bone homeostasis).

Conclusions: Altogether, these data open a new regulatory mechanism of gene expression in bone homeostasis and could direct the development of future therapeutic approaches.

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P17.37B

Human placental expression quantitative trait loci (eQTL) identified among genetic variants linked to complex traits and disease susceptibility

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Introduction: The knowledge of genetic variants shaping placental transcriptome is limited as only one whole genome analysis of placental expression quantitative trait loci (eQTLs) by Peng et al. (Human Molecular Genetics, 2017) has been published. Current study aimed to investigate the extent of placental eQTLs implicated in the programming of fetal development, postnatal metabolism and disease susceptibility.

Materials and Methods: RNA sequencing and whole genome genotyping datasets of 40 placental samples from REPROMETA cohort (Sõber et al., 2015; Kasak et al., 2015) were used for the cis-eQTL analysis in Matrix eQTL (goo.gl/wHPxxf). Selected eSNP-eGenes pairs were validated by Taqman RT-qPCR and tested for the association with birth parameters. The association testing utilized REPROMETA (n=336), HAPPY PREGNANCY (n=408) and ALSPAC (n=7669) cohorts.

Results: Analysis of cis-eQTLs identified 199 (88 independent) placental cis-eSNPs (FDR <5%). Six identified eSNP-eGenes pairs have been directly associated with blood metabolites, Parkinson's and Alzheimer's disease and colorectal cancer. Overall, 58/63 eGenes were located within 100kb of loci associated with in (auto)immunity, anthropometric parameters, blood parameters, neuropsychiatric diseases. In the REPROMETA study, a suggestive association was detected between the AA-genotype of rs11678251 (ALPG c.-318 G>A) and reduced placental, newborn's and infant's weight, but this finding did not replicate in other cohorts.

Conclusions: This is the second report cataloguing placental cis-eQTL landscape and addressing its potential link to the fetal development and postnatal disease programming.Funding: IUT34-12 (Estonian Research Council); HAPPY PREGNANCY, 3.2.0701.12-0047 (European Regional Development Fund); 102215/2/13/2 (UK Medical Research Council, Wellcome Trust), Sir Henry Dale Fellowship (WT104150).

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P17.39D

Introducing miR-885-5p as a potential biomarker for preeclampsia diagnosis: emphasizing the role of WNT signaling pathway

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Introduction: Preeclampsia is a heterogeneous condition that can be challenging to diagnose, given the wide spectrum of presentation and the current lack of a robust diagnostic test. Aberrant placental miRNA expression might be associated with preeclampsia. One of the most upregulated miRNA in the serum of PE patients is miR-885-5p. Catenin-beta (CTNNB1) is the predicted target of miR-885-5p, which has been reported to have a relatively low expression level in the placenta of PE patients. This study was conducted to determine the aberrant expression of miR-885-5p in women with preeclampsia vs. matched healthy control. Also, the target and function of the candidate miRNA was evaluated in HTR-8 and JEG-3 cell lines. Study design: Quantitative comparison of miR-885-5p expression in placenta and serum of 20 pregnant women vs. control was conducted. miRNA target analysis was done by in silico and functional assay. Expression analysis of miRNA was verified by studying cell proliferation, and apoptosis by flow cytometry, invasion, and migration by Transwell assay.

Results: miR-885-5p and its target CTNNB1 was significantly up and down-regulated in the placenta of PE patients respectively. Forced expression of miR-885-5p suppressed CTNNB1 protein expression in HTR-8 cells. The functional assay suggested that the over-expression of miR-885-5p alter invasion, migration, and cell proliferation capacity of JEG-3 cells and induce apoptosis of HTR-8 cells.

Conclusion: miR-885-5p involved in the pathogenesis of preeclampsia by targeting CTNNB1 and regulating the invasion and apoptosis of trophoblast cells. It can be suggested as a potential biomarker for risk assessment of preeclampsia.

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P17.40A
Disease interpretation of regulatory variants with GeneHancer

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Whole genome sequencing (WGS) variant analysis and interpretation requires an exploration of non-genic functional genomic elements such as promoters and enhancers, involved in development and implicated in disease. The comprehensive identification of regulatory elements and their gene targets is a significant challenge. We created GeneHancer (PMID:28605766), a regulatory element database within the GeneCards Suite (https://www.genecards.org/), with 250,000 enhancers and promoters. Information is amalgamated from ENCODE, Ensembl, FANTOM5, VISTA, dbSUPER, EPDnew, UCNEbase and GTEx. GeneHancer creates a unique non-redundant and comprehensive view of regulatory elements, including their target gene associations, transcription factor binding sites, tissue specificity and super-enhancer mapping. We recently focus on phenotype/disease annotation of such elements, based on variant-trait mappings from the GWAS Catalog, and literature-curated evidence from DiseaseEnhancer and NCBI Entrez Gene. GeneHancer is now used by a considerable fraction of GeneCards' 4 million user base to annotate non-coding variants, and was recently included as a native regulation track at the UCSC genome browser, where it is the only source for explicit regulatory regions and gene associations. GeneHancer provides an indispensable augmentation for the GeneCards' NGS disease interpretation tools: VarElect, a phenotype interpreter, and TGex, a VCF-to-report analyzer (PMID:27357693), used by major clinical sequencing centers. Non-coding variants are mapped to regulatory regions, and then prioritized with respect to diseases and phenotype keywords via direct and target gene-mediated links. Such capacities provide a comprehensive route to deciphering the clinical significance of non-coding single nucleotide and structural variations, thus helping to elucidate unsolved disease cases. Support: LifeMap Sciences grant

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P17.41B
Understanding Epigenetic relevance by evaluating Genetic underpinnings of One Carbon metabolism in Intracranial Aneurysm

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Introduction: Intracranial aneurysm (IA) leads to a devastating consequence called subarachnoid hemorrhage and it is crucial to have an early detection. Possible genetic or epigenetic biomarker identification is important to have a proper management in case of IA.

Material and Methods: Ethnically and geographically matched subject were selected and subjected to genomic DNA isolation from peripheral blood using phenol-chloroform method. Genotyping was carried out by Sanger's dideoxy method. Various statistical and computational methods like chi-square, LD analysis, Haploview were performed. Evaluation of gene expression for risk allele was carried out using In-silico approach by mining GTEx data. Functional relevance of the associated SNPs were further carried out by retrieving data from Ensembl and Haploreg to identify possible mode of impact of SNP on the regulation of the gene.

Results: *MTHFR*rs1801131 (A1286C) present in C-terminal regulatory domain, was found to be significantly associated with IA. Another variant *MTRR*rs10380 present near to FADH2 binding pocket, which is in an active site for the co-factor vit-B12 was also found to be significantly associated. *BHMT* involves with the direct conversion of homocysteine (HCy) back to methionine with the help of betaine and choline, here SNP rs3733890 was associated with Aneurysm. GTEx data for the associated alleles do indicate altered expression profile suggesting a possible accumulation of HCy.

Conclusions: Risk alleles in one carbon metabolism genes are associated with IA indicating increased HCy accumulation. Understanding this role of one carbon metabolism in IA may suggest a genotype specific vitamin intervention in IA prevention.

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P17.42C

Genome-wide circular RNA analysis of tMCAO rat model: novel insight to the neurotransmission regulation in rat brain after cerebral ischemia

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Introduction: Ischemic brain stroke is one of the most serious and socially significant diseases. The study of regulatory RNAs in ischemia has exceptional importance for the development of new strategies for neuroprotection. The circular RNAs (circRNAs) have closed structure, predominantly brain-specific expression and remain highly promising targets of research. CircRNAs can act as miRNA sponges for protection of the encoding mRNA. The work is devoted to the study of the circRNA functioning in the rat brain after temporary middle cerebral artery occlusion (tMCAO).

Materials and Methods: tMCAO model, magnetic resonance imaging, histological examination, high-throughput RNA sequencing (RNA-Seq), real-time RT-PCR, bioinformatics.

Results: Using RNA-Seq 11,134 circRNAs were analysed in the subcortical structures of the rat brain. We revealed 395 circRNAs that changed their expression (Fold change>1.5, padj<0.05) at 24h after tMCAO. They were encoded by glutamate receptor (Gria1, Grm3), catalytic (Adcy5, Ntrk2), transporter (Kcnd2, Kcnq2) and other genes associated mainly with neurotransmission signaling pathway. Bioinformatic analysis predicted circRNA-miRNA-mRNA network which may determine the neurotransmission signaling regulation for stroke. The largest number of competitive interactions with miRNAs was found between circRNAs of Marf1, Ppp4r4 and Plcb1 genes, as well as mRNAs of Stx1a and Ccnd1 genes that changed their expression after tMCAO.

Conclusion: Genome-wide circRNA profiling reveals novel insight to the neurotransmission regulation in rat brain after cerebral ischemia. We assumed that circRNAs may be key nodes of the regulation of the neurotransmission genetic response and therapeutic targets for stroke.

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P17.43D

The T1D-associated lncRNA *Lnc13* modulates pancreatic beta cell inflammation by allele-specific stabilization of *STAT1* and *STAT2* mRNA

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The majority of type 1 diabetes (T1D) association signals lie in non-coding regions of the human genome and many have been predicted to affect the expression and secondary structure of lncRNAs. However, the mechanisms by which these molecules contribute to the pathogenesis of T1D remain to be clarified.

Preliminary results of our group have demonstrated that the expression of several T1D-associated lncRNAs is modified by diabetogenic stimuli, such as viral infections and pro-inflammatory cytokines, in pancreatic β cells. Of special interest are the results obtained in the characterization of *Lnc13*, a lncRNA expressed and upregulated by viral dsRNA in β cells that harbors a SNP associated with T1D (rs917997).

Overexpression of *Lnc13* in human β cells led to an increase in STAT1/2 pathway activation that correlated with increased production of pro-inflammatory chemokines. Interestingly, when the *Lnc13* harboring the risk allele for T1D (rs917997*C) was transfected, the increase in STAT1/2 signaling was more pronounced than in cells transfected with the plasmid encoding the *Lnc13* with the protection allele (rs917997*T). In addition, the effect of *Lnc13* upregulation on chemokine production was also allele-specific.

Our studies have shown that intracellular PIC induces *Lnc13* translocation from the nucleus to the cytoplasm, increasing *STAT1/2* mRNA stability by promoting its interaction with a protein named PCBP2.

In conclusion, our results show that *Lnc13* participates in pancreatic β cell inflammation via regulation of the STAT1/2 signaling pathway, suggesting a functional effect of this lncRNA in T1D pathogenesis.

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P18
Genetic epidemiology - Population genetics - Statistical methodology - Evolutionary genetics

P18.01A
The demographic history of Afro-descendants in the Vale do Ribeira region (São Paulo, Brazil), revealed by genomic data

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Introduction: During Colonial Period, about four million Africans were brought to Brazil as slaves. Throughout this period, many runaway, abandoned and freed slaves founded small isolated rural communities, the *Quilombos*. In the state of São Paulo, most *Quilombo* remnants are located in the Vale do Ribeira region.

Objective: To shed light on the admixture dynamics of *12 Quilombo* communities in Vale do Ribeira, we used a genomics approach.

Material and Methods: 665 individuals were genotyped with ~600K SNP array (Axiom Human Origins, Affymetrix), and analyzed to estimate ancestry and effective population sizes (Ne).

Results: African, European and Native American ancestries contribute, respectively, 44%, 40%, and 16% for autosomes, 49%, 2%, and 49% for mtDNA and 32%, 63%, and 6% for the Y chromosome. This reveals directional mating involving European men with African and Native women. We further dissected timing of admixture and fluctuation in effective population sizes. Using ancestry estimates of Ne based on IBD, we found the smallest Ne ~7 generations ago, coinciding with the origin of these communities. We inferred that admixture between Africans and Europeans occurred with greatest intensity 13 generations ago (± 8-18), prior to the origin of these Quilombos. However, admixture with Native Americans occurred 7 generations ago (±3-12).

Conclusion: The *Quilombo* populations have a history of directional mating between European men with African and Native women, involving two main periods of admixture, 13 (involving Africans and Europeans) and 7 (involving Native Americans) generations ago.

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P18.02B
Digenic autosomal Alport syndrome in the context of isolated population

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Introduction: Studying population isolates in the context of genetic and phenotypic variation can provide us a unique

insight into genetic differentiation and phenotypic expressions. One of the isolated communities is the Galičnik population. It is one of the oldest villages in Macedonia, founded by the Slavic ethnic group called Mijaks around the 10th century. Among the individuals of Galičnik population, the nephropathy was detected with clinical manifestations linked to the Alport syndrome.

Materials and Methods: In total, 111 saliva samples were collected from Macedonian individuals with paternal and/or maternal origin from the Galičnik village. For the purpose of population analysis, 44 unrelated male samples were obtained from the dataset. A total of 16 Y-chromosome biallelic markers and 17 Y-STR markers were analysed using a high-resolution melt analysis and capillary fragment analysis. For detection of the COL4A3, COL4A4, and COL4A5 mutations, custom-made NGS panel was used.

Results and Conclusions: Analysis revealed two mutations, both affecting autosomal chromosome 2. First mutation was detected in exon 38 of the COL4A3 gene, resulting in an in-frame deletion of three amino acids and the second mutation in exon 13 of the COL4A4 gene, resulting in a glycine substitution within Gly-X-Y triple helical domain. Segregation analysis revealed that the two mutations were inherited together on the same chromosome, like in cis following digenic autosomal inheritance pattern. Population analysis of the Galičnik population showed a high level of genetic homogeneity within Galičnik population and strong genetic affiliations of Galičnik population with West-Slavic populations, especially the Polish population.

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P18.03C
The GenomeAsia 100K Pilot Project: Enabling Medically Relevant Genetic Discoveries Across Asia

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Introduction: Population-scale genome sequencing projects designed to lay the foundation for furthering our understanding of the genetic basis of disease are underway in the US and Europe, but large-scale and in-depth characterization of Asian genome variation has not yet been attempted despite the incredible diversity across the continent. We describe here the first stage of a project to characterize genetic variation across Asian populations with the ultimate goal of providing a rich resource for precision medicine.

Materials and Methods: In this pilot phase we focused on population isolates to capture a broad swath of genetic diversity across the continent. We have characterized the genomes of 1,739 individuals, including 1,236 newly sequenced high coverage whole genomes, representing 64 countries and more than 200 ethnic groups.

Results: We identified 63 million SNPs, 29 million of which have not been previously described, and nearly 4 million indels. The underlying samples have GIS coordinates indicating their origin, allowing variant frequencies to be displayed geographically. To evaluate the eventual value of a population-scale catalogue of Asian variants to precision medicine and molecular diagnostics, we generated pharmacogenomic predictions, identified Asian specific or enriched disease alleles and demonstrated the value of using Asian allele frequency filters in disease gene discovery such as MODY, familial disease and cancer.

Conclusions: The dataset provides resources that will greatly increase the ability to carry out genetic studies in Asian populations and provides a reference point for scaling efforts towards a much larger catalogue of Asian genetic variation.

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P18.04D
Estimated autozygosity in Norwegian family trios and its relation to the timing of birth

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Inbreeding leads to autozygosity in long segments of the genome, and increases the probability of carrying deleterious homozygous mutations with recessive effects. Because heritability of birth timing has a strong dominant component, we hypothesized that genes involved in parturition would display higher recessive effects, driving estimated autozygosity to shorten gestational duration.

We investigated runs of homozygosity (ROH) in relation to spontaneous delivery in 13000 family trios.

We estimated maternal, fetal and paternal autozygosity as the percentage of autosomal SNP mappable distance covered by ROH (F_{ROH}) and mapped ROHs to protein-coding genes by the percentage of overlapping length between ROH and gene coordinates (ROH_{gene}). We used survival analysis to estimate the effect of F_{ROH} and ROH_{gene} on time-to-spontaneous delivery in ~9000 (discovery) and ~4000 (replication) triads of Norwegian ancestry from the MoBa cohort.

While we observed a correlation between parental and fetal F_{ROH} (R= 0.14), the correlation was not detected between maternal and paternal F_{ROH} (R= 0.06). Evidence from estimated autozygosity analysis suggests an effect of maternal F_{ROH} on spontaneous delivery risk (HR, 1.008; 95%CI, 1.003-1.014), despite failing the replication attempt (1.003; 0.994-1.011). Consistent with our hypothesis, maternal ROH_{gene} mapping revealed six protein-coding genes associated with higher spontaneous delivery risk (pvalue<$7.3x10^{-6}$, no replication) and a right skewness of z-scores (>55% of genes increased spontaneous delivery risk; χ^2 pvalue<$2.2x10^{-16}$).

Estimated autozygosity highlights the role of maternal genome on, and the dominant component of gestational duration. Whether this is due to specific genes or homozygous segments spread throughout the genome remains unknown.

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P18.05A
Whole genome sequencing data increases power and precision to genome wide association studies

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Introduction: Genome-wide association studies (GWAS) have identified associations between thousands of genetic variants and human traits. However, these associations only explain a limited fraction of the heritability of most traits. Additionally, the contribution of rare variants is still largely unknown, one reason being that they usually are not tagged by common tagSNPs used in GWAS. A powerful resource for identifying trait-associated variants is by whole genome sequencing (WGS) in cohorts comprised of families or individuals from a limited geographical area.

Materials and Methods: GWAS were performed in a kinship-structured population-based cohort from northern Sweden (N=1021) with WGS data. Genetic associations were tested for 85 inflammatory biomarker proteins measured in blood plasma samples.

Results: We identified 44 independent associations, that comprised 5813 single nucleotide variant-protein associations for 42 inflammatory markers. Six of the lead variants were low frequency variants (MAF<5%), and one association with a rare variant (MAF<1%) was identified. Here, we report 19 novel regions as well as 25 loci that were not detected in the same cohort in analyses on genotyped and imputed SNPs.

Conclusions: Even in a GWA approach, we gain power and precision by using WGS data. This is suggestive of a more accurate determination of genotypes using WGS compared to imputations. However, the limited sample size did not allow for identifying further associations with rare variants.

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P18.07C
Genome-Wide Association Study of Body Fat Distribution identifies Novel Adiposity Loci and Sex-Specific Genetic Effects

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In this study, we performed genome-wide association studies (GWAS) for the distribution of body fat to the arms, legs and trunk. Fat mass for each compartment were estimated from segmental bio-electrical impedance analysis (sBIA) for 362,499 individuals from the UK Biobank. A total of 98 loci, were identified to be associated with body fat distribution, 29 of which have not previously been associated with an anthropometric trait. A high degree of sex-heterogeneity was observed and associations were

primarily observed in females, particularly for distribution of fat to the legs and trunk. Enrichment analysis indicates involvement of mesenchyme derived tissues and cell types. In conclusion, GWAS of sBIA-determined body fat distribution revealed a genetic architecture that influences the distribution of body fat throughout the human body and showed that genetic variation has a stronger influence on the proportion of fat stored in the trunk and legs in females. Determining the genetic determinants and mechanisms that lead to a favorable distribution of body fat may help in risk assessment and in identifying novel venues for intervention to prevent or treat obesity-related disease. The work was supported by grants from the Swedish Society for Medical Research (SSMF), the Kjell and Märta Beijers Foundation, Göran Gustafssons Foundation, the Swedish Medical Research Council (Project Number 2015-03327), the Marcus Borgström Foundation, The Swedish Heart-Lung foundation, and the Åke Wiberg Foundation.

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P18.08D
The human-specific *BOLA2* duplication modifies iron homeostasis and anemia predisposition

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Recurrent pathogenic copy-number variation (CNV) at chromosome 16p11.2 is mediated by human-specific duplications under positive selection. This duplication is copy-number variant and includes 3 to 8 copies of *BOLA2*, which encodes a protein involved in the maturation of iron-sulfur cytosolic proteins.

To investigate the potential human advantage of *BOLA2* duplication, we assessed hematological traits and iron-related phenotypes of 379385 controls, 89 16p11.2 deletion and 56 duplication carriers in the UKBiobank, as deletion and duplication individuals have, respectively, less and more copies of *BOLA2* than controls. We found that 16p11.2 deletion is strongly associated with anemia (18/89 carriers, 20%, P=3e-10), particularly iron-deficiency anemia. To gauge whether this phenotype was due to *BOLA2*, we collected clinical information of 16p11.2 deletion carriers with varying numbers of *BOLA2* copies, and found an association between low *BOLA2* dosage and anemia (P=1e-4). In particular, 8 out of 15 (53%) individuals with three copies needed iron supplementation and/or were anemic. To confirm our findings, we analyzed hematological traits and plasma iron level in mouse models carrying the 16p11.2 orthologous deletion and, more specifically, *Bola2*$^{+/-}$ and *Bola2*$^{-/-}$ mice. Agreeing with human data, all models showed significantly lower blood iron and hemoglobin levels and smaller red blood cells than wild-type littermates, with a linear relation with *Bola2* dosage when comparing *Bola2*$^{+/+}$, *Bola2*$^{+/-}$ and *Bola2*$^{-/-}$ mice.

Our results show that *BOLA2* participates in iron homeostasis and a lower dosage is associated with anemia. These data highlight a potential adaptive role of the human-specific expansion of *BOLA2* in improving iron metabolism.

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P18.09A
The variety of genetic variants of the *CFTR* gene according to the 2017 Register of the Russian Federation

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Objective: To determine the diversity and frequency of the identified *CFTR* pathogenic variants in the Russian Federation (RF) based on 2017 CF Registry data.

Material and Methods: Information used from the national CF Register 2017. The CF Register included data about 3096 Cystic Fibrosis (CF) patients. Genetic tests were performed in 92.4% of patients (92.6% of adults, 87.0% of children).

Results: 196 genetic variants were identified. 44 genetic variants are not described in the CFTR data bases. The ten most common genetic variants are F508del (52.8%),

CFTRdele2.3 (6.2%), E92K (3.0%), 2143delT (2.1%), 3849 +10kbC->T (2.0%), W1282X (1.9%), 2184insA (1.8%), 1677delTA (1.8%), N1303K (1.5%), G542X (1.3%). Two pathogenic alleles were identified in 80.2%, one in 16.1%, and no allele could be detected in 3.7% of patients. The proportion of F508del homozygotes was 29.6%, heterozygotes - 46.4%, genotypes without F508del - 24.0%. The distribution of variants according to the mutation types was as follows: in frame deletions/insertions prevailed (61.3%); frameshift deletions/insertions - 10.3%; missense mutations - 6% (more common in adults); nonsense mutations - 6.6% (more frequent in children); splicing disorders - 8.3% (twice as often in adults); large in/del - 7.5% (found with the same frequency in both adults and children).

Conclusion: The most common genetic variant is F508del - 52.8%. There have been identified genetic variants that are not found in international CFTR bases, their clinical significance is further confirmed. The work was performed as part of the state assignment of the Ministry of Education and Science of Russia.

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P18.10B
Phenotypic trait association study and population history inference from 90, 737 Chinese individuals

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A recent study of large scale non-invasive prenatal testing in Chinese women has yielded new insights of phenotypic trait associations and population history by extremely low coverage sequencing data. Extending to microarray data from 90,737 samples with all genders, population genetics and association studies were performed on self-reported phenotypes of both men and women. In all minorities and randomly selected Han Chinese, a principal component analysis showed similar genetic population structure with the previous study. We also replicated the private allele sharing patterns, where levels of allele sharing with CEU and ITU from 1000 genome project are higher in northwest and southwest respectively. In the genetic adaption of Chinese population, all the 14 adaptive reported SNPs are validated significantly differentiated from north to south China. And for replicated genome-wide association study results, 69.6% (32 of 46) reported SNPs are significantly associated with height, and 69.2% (9 of 13) SNPs for BMI. Our study extended the main conclusions of the previous study to all genders and showed the potential of self-reported data for population study and phenotypic association study in China.

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P18.11C
Human genetic determinants of chronic low-grade inflammation in the general population

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Chronic low-grade inflammation plays an important role in the initiation and clinical course of many complex human diseases. To better understand the contributions of human genetic variation to chronic inflammation, we performed genome-wide association studies of plasma levels of four inflammatory biomarkers (CRP, IL-1β, IL-6 and TNF-α) using data collected in the context of the CoLaus study, a large population-based cohort including >5,000 individuals in Lausanne, Switzerland. Using general linear models, we searched for associations between 9 million genetic variants and the natural log-transformed plasma levels of the four biomarkers. We identified multiple genome-wide significant associations ($p<1.25E-8$) with CRP levels at 5 previously identified genomic loci, encompassing the genes *APOE* ($p=2.01E-16$), *CRP* ($p=3.11E-12$), *HNF1A* ($p=1.83E-11$), *IL6R* ($p=2.04E-09$) and *LEPR* ($p=3.95E-09$). Interestingly, we observed that people carrying the ApoE4 genotype, known as the leading genetic risk factor for Alzheimer's disease, have lower CRP levels, suggesting that the modulating role of *APOE* variants in Alzheimer's pathogenesis is not mediated through chronic inflammation. On the other hand, we did not identify any genome-wide significant association with IL-1β, IL-6 and TNF-α levels after correction for multiple testing, which suggest that these cytokines are less tightly genetically controlled than CRP and that common genetic variants of large effect do not play a major role in their regulation. Together, the results of this study provide further insights into human genetic control of chronic inflammation, which could lead

to better disease prediction models and to the identification of potential novel targets for diagnostics or therapeutic development.

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P18.12D
Copy number variants correlate with socio-economic status

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Introduction: Socioeconomic status (SES) is a measure of an individual's or a family's economic and social status based on multiple factors including income, education, and occupation. SES is a major determinant of health and related outcomes. Lower SES confers increased risk for stroke, cardiovascular disease, hypertension, hyperlipidaemia and obesity. Early life adversity produces lasting and deleterious effects on developmental outcome. The correlation of genetic variants and SES is only beginning to be understood.

Results: We curated an anonymised database of results from over 17,000 postnatal clinical array comparative genomic hybridisation (aCGH) performed at the Manchester Centre for Genomic Medicine between 2010 and 2017. From this database we identified 473 unique cases of pathogenic or likely-pathogenic autosomal copy number variants (CNVs) with complete inheritance status (218 cases with inherited and 255 cases with *de novo* CNVs) and postcode information. We obtained indices of deprivation associated with each of these postcodes. We found that individuals with inherited pathogenic/likely-pathogenic CNVs were significantly more likely to be living in areas of higher deprivation when compared to individuals with de novo CNVs ($p_x{}^2{}_{trend} = 2.1x10^{-6}$) or with the general population of North-West England ($p_x{}^2{}_{trend} = 4.3x10^{-14}$).

Conclusions: We show that inheritance of pathogenic CNVs and lower SES are correlated. Our results demonstrate the need to quantify the secondary social and medical consequences of lower SES of individuals with inherited CNVs. These results have important implications for planning of medical and social services and provide unique insights into determinants of SES.

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P18.13A
Higher polygenetic predisposition for asthma in cow's milk allergic children

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Cow's milk allergy (CMA) is an early-onset allergy of which the underlying genetic factors remain largely undiscovered. CMA has been found to co-occur with other allergies and immunological hypersensitivity disorders, suggesting a shared genetic etiology. We aimed to (1) investigate and (2) validate whether CMA children carry a higher genetic susceptibility for other immunological hypersensitivity disorders using polygenic risk score analysis (PRS) and prospective phenotypic data. Twenty-two CMA patients of the Dutch EuroPrevall birth cohort study and 307 reference subjects were genotyped using single nucleotide polymorphism (SNP) array. Differentially genetic susceptibility was estimated using PRS, based on multiple *P*-value thresholds for SNP inclusion of previously reported genome-wide association studies (GWAS) on asthma, autism spectrum disorder, atopic dermatitis, inflammatory bowel disease and rheumatoid arthritis. These associations were validated with prospective data outcomes during a six-year follow-up in 19 patients. We observed robust and significantly higher PRSs of asthma in CMA children compared to the reference set. Association analyses using the prospective data indicated significant higher PRSs in former CMA patients suffering from asthma and related traits. Our results suggest a shared genetic etiology between CMA and asthma and a considerable predictive sensitivity potential for subsequent onset of asthma which indicates a potential use for early clinical asthma intervention programs.

P. Henneman: None.

P18.14B
Association of *AMPD1* and *CKMM* polymorphisms with physical performance phenotype of Lithuanian elite athletes

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Introduction: Adenosine monophosphate deaminase 1 (encoded by *AMPD1* gene) and muscle-specific creatine kinase (encoded by *CKM* gene) involved in regulation of muscular energy metabolism. The aim of this study was to determine the impact of *AMPD1* (c.133C>T, p.Gln45Ter, rs17602729) and *CKM* (c.*800A>G, rs8111989) polymorphisms on physical performance of Lithuanian elite athletes.

Materials and Methods: The study involved 150 Lithuanian athletes (60 females, 90 males; stratified into: endurance-oriented (n=63), sprint/power-oriented (n=65), mixed (n=22) groups) and 150 controls (60 females, 90 males, healthy unrelated non-athletes). Anthropometric measurements and muscle strength (grip strength, short-term explosive muscle power (STEMP), anaerobic alactic muscle power (AAMP)), and maximum oxygen uptake (VO_2max) were measured. Genotyping was performed by RT-PCR (for *CKM* polymorphism) and RFLP-restriction enzyme digestion (for *AMPD1*).

Results: The phenotypic measurements were significantly different and specific to each sports groups (p<0,05). The frequencies of the *CKM* genotypes were significantly different between the male and female sprint/power-oriented athletes (AA/AG/GG: 37.8/54.1/8.1% *vs* 50.0/25.0/25.0%, p=0.035). *CKM* AA and AG genotyped athletes had significantly higher STEMP than GG genotyped athletes (p=0.005). *AMPD1* CC genotyped endurance-oriented males athletes had significantly lower values of AAMP than the heterozygous athletes (p=0.04). Moreover, *AMPD1* CC genotyped endurance-oriented female had significantly higher VO_2max than heterozygous female (p=0.01).

Conclusions: *CKM* and *AMPD1* genetic variants have different effect on Lithuanian male and female physical capacity. *CKM* AA genotype confers ability to achieve better muscle efficiency in short-term, maximum-effort requiring physical activity and *AMPD1* CC genotyped athletes have better aerobic capacity.

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P18.15C
Association between *MBL2* haplotypes and dengue severity in children from Rio de Janeiro, Brazil

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Introduction: Dengue is an arthropod-borne viral disease with clinical manifestations varying from asymptomatic to severe forms including dengue shock syndrome (DSS). An increasing number of genetic studies have outlined the association between host genetic variations and dengue severity. Mannose-binding lectin acts in first line response to dengue virus (DENV). However, data regarding the association between *MBL2* gene and dengue severity are still controversial.

Materials and Methods: We have investigated the association between 7 single nucleotide polymorphisms (SNPs) at *MBL2* gene (rs7095891, rs1800450, rs1800451, rs4935047, rs930509, rs2120131, rs2099902) and dengue severity in children from Rio de Janeiro. The case group (N=87) included children with severe disease (DSS) and a control group (N=197) was selected among neighbors and cases' household members matched by age. Statistical analyses were performed using conditional logistic regression models. Proportions of European, African and Native American ancestries were also determined to adjust for confounding.

Results: No associations were observed in single SNP analysis. However, when *MBL2* SNPs were combined in haplotypes, the allele rs7095891G/rs1800450C/rs1800451C/rs4935047A/rs930509G/rs2120131G/rs2099902C was significantly associated with risk of severe dengue (OR=4.02; $_{95\%}$CI:1.23-13.09; p=0.02) as compared to the most frequent haplotype (A/C/C/A/G/T/T). A second haplotype carrying rs4935047G and rs7095891G alleles was also associated to risk (OR=1.91; $_{95\%}$CI:1.02-3.6; p=0.04).

Conclusion: *MBL2* haplotypes are associated to dengue severity in Brazilian children even after adjustment for genetic ancestry. These results reinforce the role of mannose binding lectin in immune response to DENV and suggest that variations at *MBL2* gene may help predict disease course. Financial support: CNPq, CAPES.

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P18.16D
Influence of genetic ancestry on the human serum proteome

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Background: Disease risk varies significantly between ethnicities, however, it is unknown if these observations are a consequence of environmental or genetic factors. Investigating ethnic differences within the human proteome may shed light on the impact of ancestry on health and disease.

Methods: Admixture mapping was used to explore the impact of ancestry on a panel of 237 cardiometabolic biomarkers in 2,216 Latin American participants within the ORIGIN-trial. We developed a variance component model to determine the proportion of variance explained by local ancestral differences, and applied this model in ORIGIN. Multivariable linear regression was used to identify genetic loci affecting biomarker variability between ethnicities.

Results: Variance component analysis revealed 5% of biomarkers to have a significant effect of ancestry, including C-peptide and apolipoprotein-E. We also identified 46 local ancestry associations across 40 biomarkers. Independent analyses revealed 34 of these regions were associated at genome-wide significance with their respective biomarker in either ORIGIN Europeans (n=1,931) or Latins. Finally, we found that a genetic risk score in Latins based on ancestral differences of C-peptide levels, was associated with an increased risk of diabetes (OR=4.47 per SD, 95% CI 1.70 to 11.76, p=0.002) and measures of insulin resistance.

Discussion: Our results demonstrate the importance of ancestry on biomarker levels, suggesting some of the observed differences in disease prevalence likely has a biological basis, and that use of reference intervals should be tailored to ancestry. Specifically, our results suggest a role of ancestry in insulin metabolism and diabetes risk.

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P18.17A
Increasing incidence of the Down syndrome in the Czech Republic: Long-term population-based study

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Introduction: Down syndrome is the most common chromosomal aneuploidy syndrome, therefore it is one of the main targets for the prenatal screening/diagnostics programmes worldwide. The main goal for this study was to investigate the effect of increasing maternal age (the result of the known and ongoing demographic change in the Czech Republic) on the population incidence of Down syndrome.

Methods: Data were obtained from the official National Registry of Congenital Anomalies of the Czech Republic. We evaluated the incidence of Down syndrome (ICD-10 code Q90) in the livebirths and in the prenatally diagnosed cases (time period 1994-2017). We were also investigating other related variables (like maternal age, diagnostic/screening methods). The registration process is country-wide and compulsory by national law.

Results: The overall incidence of Down syndrome in livebirths decreased from 7.79 in 1994 (relative incidences per 10.000 live births) to 3.48 in 2017 ($p<0.05$). The incidence of prenatally diagnosed (and electively terminated) cases increased rapidly from 5.35 in 1994 to 25.53 in 2017 ($p<0.05$). The average maternal age increased from 22.9 in 1994 to 30.2 in 2017.

Discussion: The overall incidence of Down syndrome in the Czech Republic is increasing (the incidence is increasing in prenatally diagnosed and terminated cases, the incidence in births is decreasing). We identified two main reasons: 1) gradual implementation of combined screening

of the first trimester; 2) rapidly increasing maternal age in the Czech Republic during the last 30 years.

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P18.18B
XRCC1 and ERCC1 DNA repair gene deficiency leads to an increase in complex conversion frequency during meiosis

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Introduction: The repair of DNA lesions, mismatches or double strand breaks (DSBs) during meiosis plays a critical role for maintaining genome stability and integrity. Meiotic recombination is initiated via programmed DSBs in hotspots, which are repaired as crossovers (COs) or non-crossovers (NCOs). Regions of heteroduplex DNA, formed during strand invasion in DSB repair, can lead to transmission biases. Of particular interest is this bias in terms of repeat instability, such as microsatellites, which are often linked to genetic disorders. Unfortunately, the association between meiotic recombination and repeat instability is not well understood.

Method: Therefore, thousands of single COs and NCOs collected via pooled sperm typing in nine different donors were analyzed for transmission biases of two different poly-A repeats: a 6A/7A and a 9A/19A.

Results: Heterologies between heterozygous poly-As strongly influenced the transmission: an insertion bias for the short heterology (6A/7A) was observed in contrast to the deletion bias for the long heterology (9A/19A). One donor was of particular interest, showing in addition 12x more complex COs (unconverted SNPs between converted ones). Whole Exome sequencing of this donor showed a missense mutation c.1196A>G at XRCC1 involved in base-excision repair and a synonymous mutation c.354T>C at ERCC1 involved in nucleotide excision repair.

Discussion: This indicates that mismatch repair has a major impact in the transmission of microsatellites during meiotic recombination and that mutations in repair genes can lead to aberrant recombination products and a massive increase in complex events.

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P18.19C
Whole exome profile of Bulgarians

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Introduction: In order to further characterize Bulgarian exome structure we have performed whole exome sequencing of healthy unrelated Bulgarian subjects and analyzed the obtained results in different contexts.

Materials and Methods: We have studied two DNA pool samples of 32 Bulgarian centenarians and 61 healthy controls (aged 18 to 30). Samples were sequenced at BGI. Statistical analyses were undertaken with the R package on frequencies of filtered variants from the present study - biallelic autosomal single nucleotide variants and gnomAD genome data. GnomAD genome data were chosen for comparison, since our results include many non-coding variants and furthermore, gnomAD exome includes exomes of 1335 Bulgarians out of 7718 non-Finish Europeans (NFE).

Results: The Bulgarian exome contains 131 private variants, with frequency >20%, not included in the gnomAD

genome database. Principal component analysis clusters Bulgarians with NFE, Finns and Ashkenazi Jews setting them apart from Americans, Africans and East Asians. The pair-wise correlation/Fst values between populations in the cluster are as follows: Bulgarians/NFE - 0.95/0.0021; Finns/NFE - 0.98/0.001 and Bulgarians /Finns - 0.95/0.0034. The greatest differences between Bulgarians/Fins (populations marking the northwest/southeast genetic gradient of Europe) are mostly due to variants in genes of the immune system; lipid, protein and carbohydrate metabolism; cell cycle and cell-cell communication.

Conclusions: The peculiarities of the Bulgarian exome will require the establishment of a Bulgarian National Genome Project for more precise characterization of the phenotypic effect of genetic variants. Acknowledgment - DN 03/7 from 18.12.2016 - National Science Fund of Bulgaria.

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P18.20D
Resolving the complex variation of the human inhibitory Fc receptor FCGR2B

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Segmental duplications in the human genome are enriched for genes involved in immunity and host defence. Characterisation of sequence variation within these regions using high throughput sequencing can be challenging due to extensive copy number variation, gene conversion, and mismapping of sequence reads to alternative paralogues. Fc-gamma Receptors (FcγRs) are a family of glycoproteins that bind to the Fc portion of the Immunoglobulin G (IgG) and mediate the response of effector cells to immune complexes. The genes encoding the low-affinity FcγRs (*FCGR2A*, *FCGR2B*, *FCGR2C*, *FCGR3A*, and *FCGR3B*) are located in an 82.5-kb segmental tandem duplication on chromosome 1q23.3 and characterized by extensive copy number and sequence variation and high sequence similarity. There is a 92%-96% sequence homology among *FCGR2A*, *FCGR2B* and *FCGR2C* genes. *FCGR2B* encodes the Fc gamma receptor IIb (FcγRIIb) (CD32B), the only inhibitory FcγR that leads to the downregulation of B-cell activation and immunoglobulin production. Genomewide association studies have highlighted variants in *FCGR2B* associated with disease phenotypes, but the context and consequence of these variants within the larger landscape of variation of this region remains unclear. Here, we assay *FCGR2B* sequence variation using targeted paralogue-specific high throughput sequencing, and place this variation in the context of recently-identified gene conversion variants, expression variation of *FCGR2B*, and GWAS association signals.

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P18.21A
Genetic epidemiological study of hereditary ophthalmic pathology in children of the Karachay-Cherkess Republic, Russia

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Objectives: Clinical and molecular genetic epidemiological study of non-syndromic hereditary ophthalmic pathology (NSHOP) is one of the promising areas of genetics due to the high fitness and survival of genotypes and the possibility of treatment and rehabilitation when the disease is detected in childhood. To our best knowledge, these studies are conducted in a limited format, within the framework of individual diseases or generalized data from individual sources.

Materials and Methods: 90,793 children from the Karachay-Cherkess Republic (KChR) were examined. 121 patients from 109 families from the KChR were included in the clinical, molecular, epidemiological analysis. Genetic epidemiological, clinical, paraclinical, molecular genetic, and statistical methods were used in the work.

Results: The total prevalence of NSHOP in child population of KChR appeared to be 1:671 children. 45 clinical and genetic forms were revealed, frequent of which are congenital ptosis (1:11342), microphthalmia (1:22685), aniridia (1:30246), congenital cataract (1:5671), retinal pathology (1:22685), optic nerve pathology (1:11342). DNA diagnosis revealed a novel nucleotide variants in the *PAX6* gene: c.607C>T, (p.Arg203Ter), c.747_750del (p.Pro250Lysfs*21) associated with congenital aniridia; in the *CHST6* gene: c.610C>T (p.Pro204Ser) and c.1124delT (p.Val375Glyfs*6) associated with corneal dystrophy; as well as in the gene *PRPF8* gene: c.428A>G, (p.Gln143Arg) associated with retinal pathology.

Conclusions: Occurrence of NSHOP in child population of KChR was 1:671. Congenital malformations and

pathology of the posterior segment of the eye are prevalent in the structure of pediatric disorders spectrum. DNA diagnosis revealed several novel variants in the genes associated with NSHOP. Supported by RFBR grant №18-015-00090.

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P18.22B
Differences in complex trait architecture between multiple ethnic human populations revealed by pathway-based epistatic interactions

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Genome-wide association (GWA) studies have identified thousands of significant genetic associations in humans across a number of complex traits. However, the vast majority of these studies use datasets of predominantly European ancestry. It has generally been thought that complex trait genetic architecture should be transferable across populations of different ancestries, but recent work has shown a number of differences between ethnic groups, including heterogeneity in both the identified causal variants and estimated effect sizes. Here, we reveal further evidence that complex trait genetic architecture is fundamentally different between human ethnic groups by jointly leveraging pathway and epistasis analysis.

Under the assumption that a given complex trait may have differential polygenic architectures across human ancestries, we hypothesize that human populations may also be enriched for differences in epistatic effects. However, since polygenic traits tend to have smaller GWA effect sizes, combining variants via pathway analysis may allow us to better reveal these signals. To accomplish this, we build on a recently published method for identifying marginal epistasis (MAPIT), moving from testing single variants to testing groups of variants for nonlinear association with a trait of interest.

We apply our new method to multiple ancestries present in the UK Biobank and explore many pathway-related interaction models. Using morphometric traits we find evidence for differential architecture among African, Indian, and British individuals. We also find evidence that these associations contain heterogeneous signals for epistatic effects. Lastly, we find preliminary evidence that complex trait regulatory architecture may be enriched for epistatic interactions.

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P18.23C
Does the doubly-weighted genetic risk score improve the prediction of type 2 diabetes in the Lifelines&Estonian Biobank cohorts?

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Introduction: It is speculated that up to half of T2D cases are undiagnosed since it mostly starts without acute symptoms. Developing a powerful tool for early detection of high-risk individuals would allow postponing or even preventing T2D. Since genetic markers are fixed for life, they have high potential for early detection. Therefore, it is important to find the optimal genetic prediction method. Our aim is to test the performance of the recently developed doubly-weighted genetic risk score (dwGRS) for incident T2D in two European cohorts.

Materials and Methods: Data from Lifelines (n=12,027) and the Estonian Biobank (n=41,107) were used. The dwGRS for T2D uses an additional weight based on the probability for the SNP to belong to the top 1,000 associated ones thus correcting for the Winner's curse bias. The additional value of dwGRS to the incident T2D prediction model was tested using survival analyses on top of predictors from the FINDRISC model. Hazard ratios and corresponding 95% confidence intervals (CI) were calculated. The net reclassification index was used to investigate the value of dwGRS.

Results: Preliminary results in Lifelines show that every standard deviation higher dwGRS increased the risk for T2D by 1.25 (95%CI 1.10−1.44) compared to 1.20 (95%CI 1.05−1.37) for the traditional GRS. Similar analyses will be applied in the Estonian Biobank.

Conclusion: The dwGRS has a significant additional value to the prediction of incident T2D. Implementation of such risk scores in personalized risk prediction could lead to early detection of high-risk individuals, resulting in better-targeted preventive measures.

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P18.25A
Evidence of thicker epidermal layer in individuals heterozygous for mutation c.-23+1G>A in *GJB2* gene (Cx26)

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Mutations in the *GJB2* gene encoding transmembrane gap junction protein connexin 26 (Cx26) are known to account for a significant proportion of recessive genetic deafness. The Cx26 expresses not only in the inner ear but also in the skin epidermis and other tissues. The high carrier frequency of the *GJB2* mutations in many ethnic populations may be explained by a heterozygous advantage of the *GJB2* mutations carriers due to their thicker epidermal layer that may provide a protective mechanism against pathogen invasion and hostile environment. We analyzed (by skin ultrasonography) the epidermis thickness in 152 Yakut individuals with different *GJB2*-genotypes ([Wt];[Wt], [Wt];[Mut], [Mut];[Mut]) for mutation c.-23+1G>A (Mut) that was found with extremely high carrier frequency (10.3%) in the indigenous Yakut population of Eastern Siberia. Individuals who were homozygous (genotype [Mut];[Mut]) and heterozygous (genotype [Wt];[Mut]) for c.-23+1G>A have the thicker epidermal layer (0.245 mm and 0.269 mm, respectively) compared with individuals with genotype [Wt];[Wt] (0.193 mm) ($p<0.05$). These data may support the hypothesis about the selective advantage of the *GJB2* mutant alleles carriers and partly explain the extremely high carrier frequency of the c.-23+1G>A mutation in Yakut population in Eastern Siberia. This study was supported by the Ministry of Education and Science of the Russian Federation №6.1766.2017, the Project NEFU M.K. Ammosov, the Programs of Bioresource collections of the FASO Russia BRK: 0556-2017-0003 and the Russian Foundation of Basic Research (grants #17-29-06016-ofi_m, #18-54-16004_NCNIL_a, #18-015-00212_A, #18-013-00738_A, #18-05-60035_Arctica).

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P18.26B
Heritability and genome-wide association study of orbital telorism

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Background: The interocular distance, or orbital telorism, is a clinically informative measure. While its extremes, hypo- and hypertelorism, have been linked to genetic disorders, little is known about the genetic determinants of interocular distance within the general population. We aimed to study the heritability and perform a genome-wide association study (GWAS) of telorism.

Methods: Telorism was defined as the distance between the eyeball center of gravity identified on cranial MRI images. First, we used a population-based and a family-based cohort studies to estimate the narrow-sense heritability (h^2) and broad-sense heritability (H^2). Second, we performed a GWAS using HRC-imputed genotypes. Models included adjustment for age, sex, height and head size.

Results: Heritability of telorism ranged between h^2=31% and H^2=74% in the fully adjusted model (Table1). GWAS of telorism identified an intronic genetic variant on chromosome 15 (rs62357458, MAF = 0.19, P-value = 5.00E-08).

Conclusion: Telorism is a heritable polygenic trait. We identified an intronic variant on chromosome 15 that was significantly associated with telorism. Replication is ongoing in ~20,000 individuals, with subsequent genetic correlation and enrichment analyses planned next. Identification

of the underlying genetics of normal variation of telorism might help us understand the pathophysiology of the related genetic disorders.

Table Narrow-sense and broad-sense heritability of orbital telorism

	Population-based study		Family-based study	
	N=3,222 unrelated individuals		N=364 with known familial relationships	
	Orbital telorism			
	h^2 (SE)	P-value	H^2 (SE)	P-value
Model 10.31 (0.10)		9.72E-04	0.76 (0.12)	1.79E-08
Model 20.32 (0.11)		9.55E-04	0.75 (0.12)	1.37E-08
Model 30.31 (0.11)		1.17E-03	0.74 (0.13)	1.00E-07

h^2: Narrow-sense heritability. H^2: Broad-sense heritability. Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex and height. Model 3: Adjusted for age, sex, height and head size. SE: Standard error.

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P18.27C
The length of the expressed 3' UTR is an intermediate molecular phenotype linking genetic variants to complex diseases

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Introduction: The investigation of the genetic determinants of intermediate molecular phenotypes can provide useful insights about the mechanisms of action of genetic variants that have been identified by genome wide association studies (GWAS). We propose a computational strategy to discover genetic variants affecting the relative expression of alternative 3' untranslated region (UTR) isoforms, generated through alternative polyadenylation (APA), a post-transcriptional regulatory mechanism known to have relevant functional consequences.

Material and Methods: We analyzed whole genome sequencing and RNA sequencing (RNA-Seq) data that were obtained for 373 European individuals (GEUVADIS dataset). RNA-Seq data were used, together with an annotation of alternative 3'UTR isoforms, to compute the expression ratio between the short and long isoform (m/M value) of each gene in each individual. Then, the association between the m/M values of a gene and the genotype of the individuals for each in-cis genetic variant was evaluated by linear regression.

Results: We identified 2,530 genes with alternative polyadenylation quantitative trait loci (apaQTLs) and we subsequently performed a deep investigation of the possible mechanisms of action of these variants. In addition, we observed that apaQTLs are significantly enriched in GWAS hits, in particular those associated to immune-related and neurological disorders.

Conclusions: Our results suggest that genetic variants have a widespread effect on the relative expression of alternative 3'UTR isoforms in human. In addition, they point to an important role for genetically determined APA in affecting predisposition to complex diseases and suggest new ways to extract functional information from GWAS data.

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P18.28D
Genome-wide association analysis of 350,000 Caucasians from the UK Biobank identifies novel loci for asthma, hay fever and eczema

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Introduction: Even though heritability estimates suggest that the risk of asthma, hay fever and eczema is largely due to genetic factors, genetic variants identified previous studies have failed to explained a major part of the genetics behind these diseases.

Material and Methods: In this GWAS, we include 346,545 Caucasians from the UK Biobank to identify novel loci for asthma, hay fever and eczema. We further investigate if associated lead SNPs have a significantly larger effect for one disease compared to the other diseases, to highlight possible disease specific effects.

Results: We identified 141 loci, of which 41 are novel, to be associated ($P \leq 3x10^{-8}$) with asthma, hay fever or eczema, analysed separately or as combined phenotypes. The largest number of loci were associated with the combined phenotype (asthma/hay fever/eczema). However, as many as 19 loci had a significantly larger effect on hay fever/eczema-only compared to their effects on asthma, while 24 loci exhibited larger effects on asthma compared with their effects on hay fever/eczema. At four of the novel loci, *TNFRSF8, MYRF, TSPAN8*,and*BHMG1*, the lead SNPs were in LD (> 0.8) with potentially casual missense variants.

Conclusions: Our study shows that a large amount of the genetic contribution is shared between the diseases. Nonetheless, a number of SNPs have a significantly larger effect on one of the phenotypes suggesting that part of the genetic contribution is more phenotype specific.

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P18.29A
Cross-trait analysis of brain volume and intelligence identifies shared genomic loci and genes

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Introduction: The phenotypic correlation between human intelligence and brain volume (BV) is considerable ($r \approx 0.40$) and has been shown to be due to shared genetic factors. Large-scale GWAS for these traits have identified many novel loci and genes that explain their genetic architecture. However, it is unknown which shared loci and genes explain the genetic overlap between these traits.

Materials and Methods: To further examine the nature of this correlation, we present genomic analyses of the genetic overlap between BV and intelligence using genome-wide association study (GWAS) results. We conducted a GWAS meta-analysis of a collated dataset on measurements of BV (N=54,407), followed by extensive functional annotation and gene-mapping, and study of the overlap with GWAS results of intelligence (N=269,867) performed by our group.

Results: GWAS meta-analysis identified 35 genomic loci, 362 genes and 23 biological pathways for BV. We estimated the genetic correlation between BV and intelligence of. r_g=0.24. This overlap is driven by physical overlap in 5 genomic loci. By using several gene-mapping strategies (including FUMA), we observed 67 overlapping genes for both traits. Of these, several are involved in cell-cycle regulation (*ERBB3*, *USP19*), neuron morphology (*INA*), or genes involved in neurodegenerative disorders (*MAPT*). We highlight cortical areas of the brain where these genes are expressed.

Conclusions: These results provide new information on the genetics of BV and insight into the biology of its shared genetic aetiology with intelligence. These results illustrate how large-scale GWAS and subsequent gene-mapping aid in understanding the genetic cross-section between seemingly distinct traits.

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P18.30B
Prevalence and distribution of human knockouts in Middle Eastern populations with a high rate of consanguinity

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Introduction: Homozygous loss of function (HLOF) variants, or naturally occurring "human knockouts (KO)", provide an invaluable opportunity to gain scientific and clinical insights into gene function, as well as an inventory of human genes that are dispensable in healthy individuals. Genomic data obtained from consanguineous and bottlenecked populations, as found in the Middle East, are particularly useful for HLOF analyses, as they are enriched in homozygous variants.

Materials and Methods: Whole exome sequencing data from 2663 Middle-Eastern individuals were queried for HLOF variants. Stop-gain, high-quality frameshift, and splice-site variants, with a read depth of 15X or greater in canonical transcripts, were included. No filter was placed on minor allele frequency.

Results: Querying HLOF variants in 2663 exomes (2030 affected, 633 healthy) yielded 107,393 variants, representing 1155 unique variants in 952 genes. These included 378 stop-gain, 520 frameshift, and 257 splice-site variants. An average of 40.33 HLOF variants were identified per exome. 413/952 (43.4%) of the unique genes identified in this study were previously reported as human KO genes. Rare HLOF variants in affected individuals included previously published disease-associated genes, such as *WDR16*, *SNX10*, *PLD1*, *CARD11*, and several candidate disease genes.

Conclusion: Rare HLOF variants in affected individuals are potentially disease-causing variants, whereas HLOF variants in healthy individuals may have beneficial effects and thus are potential drug discovery targets. Reassuringly, only 43.4% of the genes identified in this study overlap with previously reported human KO genes, underscoring the potential utility of the current research.

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P18.31C
Heritability estimates in recently admixed populations: insights from the Greenlandic population

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Finding an efficient framework for estimating narrow-sense heritability in admixed populations with high relatedness remains an open question. We used extensive simulations to evaluate the performance of existing classical and linear mixed model frameworks in estimating narrow-sense heritability in two population-based cohorts from Greenland and compare it to data from unadmixed individuals from Denmark. When our analysis focused on Greenlandic sib pairs, the model with two relationship matrices, one capturing identity by state and one capturing identity by descent, returned heritability estimates close to the true simulated value, while using each of the two matrices alone returned more biased estimates. When phenotypes correlated with ancestry, heritability estimates were inflated. Based on these observations, we propose a PCA-based adjustment in order to recover the true simulated heritability. Finally, we estimated heritability of ten quantitative traits from the two Greenlandic cohorts and report differences such as lower heritability for height in Greenlanders compared to Europeans. In conclusion, total narrow-sense heritability in recently admixed populations is best estimated using a mixture of genetic relationship matrices on individuals with at least one first-degree relative included in the sample.

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P18.32D
High confidence HLA resource based on 1000 Swedish genomes

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Introduction: Recently genome variation from 1000 individuals of Swedish ancestry was reported based on short read 30x Illumina NGS data. From this curated dataset, a high confidence (HC) HLA (classical eight genes: *A*, *B*, *C*, *DPA1*, *DPB1*, *DQA1*, *DQB1*, *DRB1*) resource was constructed for the use of the wider research community.

Materials and Methods: A combination of software (HLA-VBSeq, HLAscan, OptiType, SNP2HLA) was used to call HLA alleles from BAM files, followed by concordance to determine the final HC set (minimum of n-1 software calls the same four digit allele). The population frequency of HC alleles was compared to published Swedish lab typed values where available, and eight gene haplotypes contrasted to Caucasian cohorts.

Results: The complete set of classical eight HC alleles were typed in 608 samples, improved to 926 when considering only Class I alleles (*A*, *B*, *C*). This was due to a combination of software and IMGT/HLA availability. Comparison with the lab typed cohort showed the HC alleles reflected expected values (r^2=0.81-0.99, five genes), with minor differences perhaps due to reference versions. Four of the top five haplotypes found in the HC set were also top ranked in the Caucasian cohorts.

Conclusion: The Swedish HC HLA dataset adds to the growing set of population resources available to interrogate variation within the MHC. The HC allele frequencies (and individual calls at request), plus calls from each software, will be made available via https://swefreq.nbis.se/.

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P18.33A
The relevant role of Italian genetic isolates for the study of Human Knockouts

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Introduction: Rare (MAF<1%) Loss of Function (LoF) variants have been characterised with huge study effort on large cohorts and isolated communities, thanks to their enrichment for biallelic knockout events. A sub-category of LoF variants is represented by human knockouts (HKO).

Materials and Methods: Low coverage whole genome sequence data of 946 samples from three Italian isolated cohorts was performed. All the LoF variants with at least one homozygous carrier in the whole dataset and a CADD score>=20 or "not available" were considered.

Results: We obtained a total of 506 variants, 205 classified as total LoF and 301 as partial LoF (i.e. predicted as LoF only in some gene transcripts). We selected LoF variants from 30 interesting genes involved in mendelian

diseases. A rare partial LoF mutation in *FANCL* (associated with Fanconi anaemia) was detected in homozygosis in one individual and in heterozygosis in four additional subjects, from the same isolated village. We further investigated the subjects' clinical history: the *FANCL* KO subject shows a long history of head and neck cancer without the classical Fanconi haematological features, while the *FANCL* carriers are healthy.

Conclusions: HKO study has already shown great potential despite being in its very early stages. Thanks to our large cohort of genetic isolates, analysed by WGS, we highlighted the role of one LoF variant in *FANCL*, particularly enriched in one of our villages and not being associated to the expected phenotype. Functional studies to evaluate the potential phenotypic consequences of this variant will be performed.

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P18.34B
Trait aggregation analysis in families with large pedigrees: techniques, assessment and examples

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Introduction: In epidemiological studies, familial aggregation (FA) analysis provides an important early means for determining promising phenotypes, which may be influenced by genetic factors. Availability of comprehensive ancestral information is especially valuable for detecting remote relationships in otherwise disconnected families. We have implemented and assessed FamAgg, an R package for detecting related individuals affected by some trait in large pedigrees. We furthermore demonstrate its capabilities by examining traits in the context of the Cooperative Health Research in South Tyrol (CHRIS) study.

Materials and Methods: In order to assess the ability to correctly detect FA, we define families affected by a Mendelian trait (cases) and challenge five different FA methods to identify these families within a large set of pedigrees with randomly chosen affected individuals (controls). Method performance evaluation is based on varying trait prevalence and penetrance, and on the number of affected generations within the case families. The CHRIS study is a population-based study with pedigree data on >10,000 participants carried out in an alpine valley in the North of Italy. Here, participants have been investigated for FA for ~80 traits.

Results and Conclusions: Tests performed equally well for cases with low prevalence, high penetrance and many generations of affected individuals. We were able to identify tests with superior performance under more challenging conditions (e.g. 6.25% trait prevalence and 60% penetrance in three generations of affected individuals) and give recommendations on test usage and interpretation. Based on these observations, we discuss examples from the CHRIS study.

Availability: http://www.bioconductor.org/packages/FamAgg

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P18.35C
No effects of high-resolution population-specific genetic map on downstream genomic analyses

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Introduction: Recombination is an important part of meiosis as it facilitates chromosomal aggregation. Founder population size, demographic changes like multiple bottlenecks or rapid expansion can lead to variation in recombination rates across different populations like in the case of Finland. Previous research has shown population specific settings has a significant effect on downstream population genomic analysis like haplotype phasing, genotype imputation and association.

Materials and Methods: Here, we examined high-resolution recombination rate variation at 10 and 50kb scales using deep whole genome sequences (20-30X coverage) of 55 trios all over from Finland. We then tested the downstream effects of the population-specific effective population sizes and lower recombination rates in statistical phasing, genotype imputation and disease association mapping in Finns in comparison with non-Finnish Europeans.

Results: Population-specific effective population sizes were found to have no effect in haplotype phasing (switch

error rates ~ 2%) and average imputation concordances (97-98%). Similarly, we found no effect of population-specific recombination maps in phasing with comparable switch error rates (SER) across all autosomes. Downstream population genetic analyses depend on population-specific contexts like appropriate reference panels and their sample size but not on recombination maps or effective population sizes.

Conclusions: Currently available HapMap recombination maps seem quite robust for population-specific phasing and imputation pipelines. Grants: Academy of Finland (251217 & 255847), Center of Excellence for Complex Disease Genetics, Finnish Foundation for Cardiovascular Research, Biocentrum Helsinki & Sigrid Jusélius Foundation grants to S.R. FIMM-EMBL doctoral funding to S.H. and Academy of Finland Postdoctoral Fellowship to I.S. (298149).

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P18.36D
Genetically predicted gene expression reveals candidate genes for Inflammatory Bowel Disease risk

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Introduction: Genome-wide association studies have identified a large number of genetic variants associated to complex diseases. However, most of these variants lay in intronic or intragenic regions and do not provide a clear target gene. Expression quantitative trait loci (eQTL) studies and transcription-wide association analyses (TWAS) may contribute to explain the observed associations between genetic regions and complex diseases such as Crohn's disease and ulcerative colitis. In this study, we conducted a summary-based TWAS for Inflammatory Bowel Diseases (IBD) risk using RNA-seq data form a largest set of colon tissue biopsies from healthy volunteers.

Materials and Methods: Colon tissue biopsies were obtained from 191 healthy volunteers at colonoscopy. Genetic predictive models for the expression of ~6,000 genes were obtained through penalized elastic-net models using PredXcan software. Subsequently, summary-TWAS was performed combining genetic prediction models and genome-wide association results for IBD risk published by the International IBD Genetics Consortium.

Results: TWAS analysis identified one novel IBD locus association (INPP5E gene at 9q34.3 region; P= 1.2x10-19) and eleven new candidate genes in known GWAS loci (P<1x10-8). We envisage enlarging the analyzed data set to nearly 500 samples during the following months.

Conclusions: Our findings help to explain observed association results for diseases affecting colon tissue and provide target genes for further functional analyses. Grant references: NIH/NCI CA143237; NIH/NCI CA204279; NIH/NCI CA201407; ISC III - FEDER PI14-00613; CIBERESP CB07/02/2005; Catalan Government DURSI 2014SGR647; EU H2020 - MSC grant No 796216. FPU16/00599.

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P18.37A
Estimating additive and non-additive genetic components from low-depth sequencing data

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A variety of methods for estimating coefficients of pairwise relatedness from genotype data have been proposed. For each pair of individuals, this allows estimates of both their kinship coefficient φ and their probability ψ of sharing two alleles Identical-By-Descent. Estimating relatedness is an important step for many genetic study designs. For instance, estimates of φ and ψ are necessary to assess the respective roles of additive and non-additive genetic components in the study of complex traits. However, when dealing with low-depth sequencing or imputation data, individual level genotypes cannot be confidently called. To ignore such uncertainty is known to result in biased computations. Accordingly, methods have recently been developed to estimate kinship from uncertain genotypes. Here, we make an extension to include non-additive variance by deriving orthogonal genetic components from uncertain genotype data and proposing new estimators for both the coefficients φ and ψ. By construction, our estimators are robust to departures from Hardy-Weinberg principles, and thus could be of interest for samples that present inbreeding or

population structure. For an additional application, the orthogonality of the components allows for association testing of non-additive genetic effects without having to call genotypes. We have simulated low-depth genetic data for a sample of individuals with extensive relatedness by using the complex pedigree of the known genetic isolates of Cilento. Though this simulation, we explore the behaviour of our estimators, demonstrate their properties, and show potential advantages over alternative methods.

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P18.40D
Variants in genes related to telomere maintenance in Bulgarian centenarians

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Introduction: The Bulgarian genome has been largely studied in the postgenomic era. The GnomAD_exome database includes 1335 Bulgarian exomes and a series of international research studies involve Bulgarian exomes and genomes. In this study we further characterize genomic variations in centenarians, focusing on variants in genes related to telomere maintenance. Telomeres are complex DNA-protein structures that form protective caps at the end of eukaryotic chromosomes. They prevent chromosome degradation and form part of cellular mechanisms responsible for maintaining genomic integrity and cell longevity.

Materials and Methods: Case-control WES pool analysis of 32 centenarians and 61 young healthy controls and WGS was performed in 16 centenarians.

Results: After quality filtering a total of 610 variants were found in 82 genes known to be involved in regulation of telomere maintenance. 22 variants were found to be more prevalent in Bulgarian centenarians than in controls and for one of them (rs75803132) the P value was below $5.0x10^{-8}$ (MAF G=0,267 in BG centenarians versus MAF G=0,07 in controls). The variant rs75803132 (NM_003668.3:c.661-63A>G) in intron 8 of the gene *MAPKAPK5* has been observed at MAF 0,076 (GnomAD_genome NFE) and seems to be enriched in Bulgarian centenarians. Mitogen-activated protein kinase-activated protein kinase 5 acts as a tumor suppressor by mediating Ras-induced senescence and phosphorylating p53/TP53.

Conclusion: This pilot case-control study reveals new insights in complex genetic background of human longevity. Acknowledgment to DN 03/7 from 18.12.2016 - National Science Fund of Bulgaria

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P18.42B
MR-link: Identifying known and novel causality from gene expression to complex traits, while accounting for pleiotropy and linkage

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Mendelian Randomization (MR) is a widely used method to identify causality from genetic data, but its application to gene expression traits is challenging. Widespread pleiotropy and linkage between expression quantitative loci (eQTL) often result in violations of the assumptions behind MR. In simulations, existing MR methods demonstrate uncalibrated false positive rates (FPR) or low power to detect causality.

Here, we introduce MR-link, a novel two-sample MR method that incorporates variants in linkage disequilibrium with conditionally independent (CI) eQTL, to correct for linkage and unobserved pleiotropy. In simulations, MR-link has consistently calibrated FPR (median: 0.05) and higher power over other tested methods (max: 0.96), even when only a single CI eQTL variant is detectable.

We have now applied MR-link to LDL-cholesterol (LDL-C) measurements, joined to eQTL datasets derived from blood (2), liver, and coronary artery tissue. This identified 25 genes causal to LDL-C, including the known and validated *SORT1* transcript, along with genes that were missed by genome-wide association studies on lipids but nonetheless validated in-vivo. For example, MR-link implicates *NEGR1*, involved in the lipid storage disorder Nieman Pick disease as well as *DEPP1* expression, lowering insulin levels, which consequently affects lipids. Beside genes with known biological function, MR-link identified 15 novel genes causal to LDL-C levels, interesting candidates for follow-up experiments.

These results demonstrate that MR-link successfully identifies known causal relationships between gene expression and complex traits while also revealing novel causal genes that may further improve our understanding of the etiology of complex traits from observational data.

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P18.43C

Causal relevance of obesity on the leading causes of death in women and men: A Mendelian randomization study

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Introduction: Obesity traits have been causally linked with cardiometabolic disease risk, but the extent to which obesity traits affect risk of other leading causes of death has not been comprehensively evaluated. It is also unclear if any such causal effects differ between men and women.

Materials and Methods: We constructed sex-specific genetic risk scores for the obesity traits: body mass index (BMI), waist-hip-ratio (WHR), and WHR adjusted for BMI, including 565, 324 and 338 genetic variants, respectively. Using Mendelian randomization, we investigated for associations between these obesity traits and leading causes of mortality from non-communicable diseases in the UK Biobank. Sex-differences in the effect estimates were assessed using Cochran's Q test (P_{het}).

Results: Up to 194,697 men and 227,717 women were included, with mean (SD) age 57.0 (8.1) and 56.6 (7.9) years, BMI 27.9 (4.2) and 27.0 (5.1) kg/m^2 and WHR 0.94 (0.07) and 0.82 (0.07), respectively. Obesity traits causally increased the risks of coronary artery disease, stroke, chronic obstructive pulmonary disease (COPD), lung cancer, type 1 and type 2 diabetes (T2D), and acute and chronic renal failure. BMI increased T2D risk more in women than in men ($P_{het}=5.1\times10^{-6}$), whereas WHR increased risks of COPD ($P_{het}=5.5\times10^{-6}$) and chronic renal failure ($P_{het}=1.3\times10^{-4}$) more in men than in women.

Conclusions: Obesity traits increase the risk of the majority of the leading causes of mortality. There are sex-differences in the effects of obesity traits on COPD, T2D, and chronic renal failure, which may have implications for health policy and provision of health services.

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P18.44D

Prognostic value of expression quantitative trait loci in multiple myeloma prognosis

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Introduction: Owing to many advances in therapy during the last decade, the survival of multiple myeloma (MM) patients improved considerably, although it remains an incurable disease. Gene expression profiling (GEP) is widely used for tumor classification and survival prediction and can effectively identify patients who can benefit from particular types of therapy. A recent study has identified a GEP related to the response of MM patients to the first line treatment based on bortezomib, thalidomide, and dexamethasone (VTD). The variability of gene expression depends in part on germline genetic polymorphisms. Germline variants called expression quantitative traits loci (eQTLs) can affects gene expression. If there are eQTLs for the above mentioned GEP, they could be used as prognostic marker in MM.

Materials and Methods: We genotyped 6 expression quantitative trait loci (eQTLs) in *ENTPD1, CCND1, CCND2, ARK3, HELLS*, and *ACTR2* genes in 1284 MM cases from the International Multiple Myeloma rESEarch (IMMEnSE) consortium, and analyzed their involvement in progression free survival (PFS), overall survival (OS) and response to first line therapy.

Results: Carriers of the minor (C) allele of *ENTPD1*-rs2153213 showed a consistent better survival (PFS: HR=0.59, 95%CI=0.38-0.91, p=0.018; OS; HR=0.7, 95%CI=0.54-0.94,p=0.017).

Conclusion: According to GTEx the C allele of rs2153213 is associated with decreased level of *ETPD1*. Lower levels of *ENTPD1* have been suggested to be involved in immune modulation response against several cancers leading to a better outcome. This may explain our finding and in part the variability of MM patient's survival.

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P18.45A
Genetic predisposition to co-morbid depression and anxiety in diabetes using multi-omics framework approach

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Introduction: Depression and anxiety are highly prevalent in individuals with type 2 diabetes (T2D), affecting quality of life and well-being. Epidemiological studies suggest shared aetiology between these conditions. Genetic variants, reportedly associated with them, affect lipid metabolism, cell proliferation, immune and inflammatory response, and oxidative stress, thus suggesting shared pathophysiological processes. However, the phenotypic variance responsible for T2D and depression captured by genome-wide association studies (GWAS) explains only ~5% of susceptibility to these conditions. We aimed to identify genetic factors contributing to their co-morbidity using multi-variable analytical framework.

Methods: We analysed data from population-based Northern Finland birth cohort using 46 years-old clinical examination from 3,597 participants, Haplotype Reference consortium imputed genome-wide Illumina HumanCNV370DUO platform data was quality controlled providing >10M autosomal SNPs for analysis. Using SCOPA software, we performed the multiple-phenotype GWAS (MP-GWAS) as linear combination of residuals for T2D, anxiety (Generalized Anxiety Disorder 7-item Scale)

and depressive symptoms (Beck Depression Inventory) score, obtained after adjusting for sex and three principal components to control for population structure.

Results: Three loci, at *MICAL2* (rs10765927), *INPP5K* (rs145536147) and *ZNF599* (rs7259475) reached genome-wide significance ($P<5\times10^{-8}$). Expression of target genes at these loci, involved in cell growth, insulin metabolism and transcriptional regulation, takes place primarily in the brain and is decreased in presence of depression. rs10765927, rs145536147 and rs7259475 were associated in GWAS with neuroticism, Parkinson's and Alzheimer's diseases, respectively.

Conclusion: The results of this MP-GWAS provide first evidence for a shared aetiology between T2D, depressive symptoms and anxiety.

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P18.46B

Implementation of a scalable framework for the identification of regulatory variants at non-coding risk loci: Follow-up of GWAS findings in orofacial clefting

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GWAS have yielded unprecedented insights into the genetic etiology of multifactorial disorders. Main outcomes were that associated common risk variants are primarily located in non-coding regions, have low to moderate effect sizes, and show extended stretches of LD. For functional translation of these findings, approaches such as eQTL analyses are often applied, but this approach is restricted to traits for which disease-relevant cell types/tissues are available.

An alternative approach is via the identification of rare, deleterious mutations in individual families, and/or the increased burden of low-frequency variants in affecteds. As whole-genome sequencing is currently too expensive for large cohorts, targeted approaches (e.g., single-molecule molecular inversion probes (smMIPs)) have emerged as strong alternative. Selection criteria for candidate regions include integrated functional and genomic data.

In this study we applied this strategy to non-syndromic cleft lip with/without cleft palate (nsCL/P), a frequent birth defect with multifactorial etiology. We selected three risk loci that had shown association with nsCL/P in previous GWAS ($P<10\text{-}05$) and identified twelve candidate elements in the credible SNP regions, based on functional data from neural crest cells and craniofacial tissue. SmMIPs-based resequencing of these regions in 1,061 nsCL/P cases and 1,591 controls yielded 1,835 variants with a MAF<1%. These variants are currently analyzed both individually and in aggregate (burden analyses). Furthermore, different non-coding variant annotation tools are evaluated, and pedigree analyses will be performed. Our framework is largely scalable and can be extended to other disorders where access to relevant tissue is similarly difficult.

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P18.47C

Population-based assessment of the phenotypic profile of Melanocortin-4 Receptor mutation carriers

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Introduction: The hypothalamic leptin-melanocortin pathway constitutes the primary central control mechanism for eating behaviour. Rare mutations in *MC4R* - the commonest monogenic cause of obesity - have been mainly discovered in severely obese individuals, and the phenotypic profile of mutation carriers not ascertained for obesity is unknown. Thus, our understanding of the penetrance and expressivity of *MC4R* mutations in the general population is very limited.

Materials and Methods: Data from 329,419 unrelated White British individuals in the UK Biobank were analysed to compare anthropometric and metabolic-related

phenotypes between carriers of rare, complete loss of function (cLOF) MC4R mutations and non-carriers.

Results: The prevalence of *MC4R* cLOF mutations was 0.08%. The 276 carriers differed from 314,2017 non-carriers in: height (P=0.033), BMI (P=1.20E-05), WHR (P=0.016), various measures of body fat and muscle mass, and basal metabolic rate (P=3.12E-07) after adjustment for age and sex. Since only 31% of carriers were obese, and 25% were within the "healthy" BMI range, we sought to investigate genetic factors influencing penetrance. GRS for BMI did not appear to modulate penetrance. Co-carriage of the protective V103I *MC4R* variant moderated the effect the cLoF mutation, such that 50% of cLoF carriers, who also had V103I had normal BMI compared to 25% without V103I.

Conclusions: Our appreciation of phenotypic variability among *MC4R* cLOF mutation barriers is compromised by ascertainment bias. An unexpectedly high proportion of mutation carriers in the UK Biobank are not obese. This may be at least partly due to epistatic effects.

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P18.48D

Does genetic predisposition to higher fat expandability attenuate the impact of weight gain on cardiometabolic risk?

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Introduction: Obesity is linked to cardiometabolic comorbidities, but many obese, often called "metabolically healthy obese" (MHO), seem protected. Recent studies suggest that genetic variants associated with higher "fat expandability" contribute to this paradox by higher storage of metabolically inert, subcutaneous fat. Whether these variants attenuate the impact of long-term weight gain on cardiometabolic risk remains unknown.

Objective: We examined whether a "fat expandability" genetic risk score (GRS) attenuates the detrimental impact of weight gain on cardiometabolic risk during a 5-year follow-up. We also examined whether the GRS predicts MHO baseline status and the persistence of such status.

Methods: The GRS was constructed by combining 24 genetic loci robustly associated with increased BMI but decreased waist-hip-ratio. The interactions of the GRS*weight gain on quantitative cardiometabolic traits and associations with MHO status were examined in 5,348 participants of the Danish Inter99 cohort. MHO was defined as BMI ≥25 kg/m^2 in the presence of none or only one component of metabolic syndrome.

Results: The GRS attenuated the impact of weight gain on fasting insulin by 0.03 pmol/L per allele per each kg of body weight gained during the follow-up ($P_{interaction}$=0.031). The GRS was associated with higher odds of being MHO at baseline (OR=1.04 per allele, P=0.0002) but did not reduce the odds of converting from MHO to a metabolically unhealthy state by 5-year follow-up (OR=1.00 per allele, P=0.679).

Conclusion: Genetic predisposition to higher "fat expandability" attenuates the impact of weight gain on insulin resistance.

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P18.49A

Epigenome-wide association study of change in blood metabolite and glycaemic trait levels and in obesity measures from young- to middle adulthood in 595 Northern Finland Birth Cohort 1966 participants

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A number of associations between adiposity, metabolite and glycaemic trait levels and DNA methylation (DNAm) have been reported. However, the relationship between longitudinal changes in these phenotypes and differential methylation is underexplored. We assessed associations between DNAm and change over time in 228 blood metabolomics-based variables and waist/hip ratio; body mass index (BMI); fasting blood plasma glucose (FG), blood insulin, and serum triglyceride and cholesterol levels.

For 595 non-diabetic individuals from the Northern Finland Birth Cohort 1966 for whom phenotype data were available at both ages 31 (T1) and 46 (T2) as well as concurrent blood DNAm data at T2, we calculated the average change in phenotype value per year between T1 and T2. We used our methylSCOPA software, which can also perform multi-phenotype epigenome-wide association study

(EWAS), for single-phenotype EWAS of change residuals – corrected for sex – for each phenotype versus DNAm for 832,569 markers on the Illumina (San Diego, CA, USA) MethylationEPIC BeadChip. We quality-controlled, residualized, and normalized the DNAm data, and mapped genomic locations to CGCh37/hg19.

Specifically, for BMI change we detected epigenome-wide significant associations ($P<1\times10^{-7}$) at established loci *PHGDH* (cg14476101|chr1:120,255,992; β=-0.05, SE=9.1×10^{-3}) and *SLC43A1* (cg11376147|chr11:57,261,198; β=-0.01, SE=2.6×10^{-3}), and for FG change at cg20367077 (chr11:19,224,117; β =0.11, SE=0.02) annotated to *CSRP3*, which has a role in obesity-induced insulin resistance in murine and human skeletal muscle.

We implemented a novel method to detect associations between change in phenotype over time and DNAm and provide account of its use for a range of phenotypes.

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P18.51C

Variants within *OCA2* and *HERC2* genes associated with eye color and iris features in a Brazilian admixed population sample

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Introduction: *OCA2* and *HERC2* are considered the main genes associated with the normal variation of eye pigmentation in humans. We sequenced their regulatory and coding regions, associating genetic polymorphisms with qualitative and quantitative measurements of eye color, as well as the presence or absence of iris features.

Materials and Methods: A sample of 340 individuals from São Paulo State, Brazil, was stratified according to eye pigmentation and presence or absence of iris features. DNA libraries were prepared using Haloplex (Agilent) and sequenced at the MiSeq platform (Illumina). CutAdapt, BWA and GATK were used for trimming, alignment and genotype calling, respectively.

Results: 170 polymorphisms were called and eleven were associated with one or more qualitative and quantitative eye color phenotypes, as well as with iris features. The *HERC2* rs12913832 polymorphism was associated with the largest number of phenotypes, with its "G" allele associated with blue/green eyes. Additionally, this polymorphism was associated with iris features for the first time, such as Wolfflin's nodes ($p = 1.0\text{x}10^{-4}$), contraction furrows ($p = 5.0\text{x}10^{-4}$) and iris pigmentation spots ($p = 0.0013$). The *HERC2* polymorphism rs58358300 was associated with sclera pigmentation ($p = 1.0\text{x}10^{-4}$), hitherto not reported.

Conclusions: We identified previously unknown associations of polymorphisms with some eye pigmentation characteristics, which reinforces the need for studies in highly admixed populations such as the Brazilian, which presents favorable conditions for the identification of new variation sites that may have a functional effect on pigmentation genes. Grants and fellowships: CAPES (88881.197124/2018-01), FAPESP (2013/154470) and CNPq/Brazil (448242/2014-1 and 309572/2014-2).

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P18.52D

Accounting for cryptic relatedness between subjects with no genotype data across families

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Complex traits continue to provide challenges for identifying genes responsible for variation in disease risk. Genome sequencing has become inexpensive and fast. This provides opportunities for investigation of rare variation. In the search for disease-associated rare variation, family-based designs have again become common, because high-penetrant genotypes segregate in pedigrees.

Association testing is typically used to identify association with rare variants. When related subjects are included

in the sample, a kinship matrix must be used to account for relatedness between subjects. Relationships may be known from the pedigree structure, or can be inferred using observed genotypes. For subjects without genotype data, however, no approach is able to infer relatedness.

Pedigree-based imputation increases the sample size and thus association power, and provides genotype probabilities in subjects without genotype data. If phenotypes are available for such subjects, including them in the analysis is likely to maximize power to detect association between genotypes and phenotype. However, knowing the relationship between such subjects and the remaining ones, especially across pedigrees, is crucial to control type 1 error.

Here, we propose a solution for inferring cryptic relatedness between subjects with complete missing genotypes. Our approach uses GIGI to estimate the probabilities of missing genotypes, and integrates them in an Expectation-Maximization approach to estimate kinship coefficients. Through simulation, our approach succeeds to infer many types of relationships with relatively high confidence. It yields an average kinship estimates of 0.19, 0.1, and 0.05 for an underlying kinship of 0.25, 0.125, and 0.0625, respectively, for subjects without genotype data.

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P18.54B
Discovery of novel Viking sequences in Swedish genomes

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Abstract Novel sequences, not present in the human reference genome, are abundant and remain largely unexplored. Here, we utilize *de novo* assembly to study novel sequences present in 1000 Swedish individuals first sequenced as part of the SweGen project revealing a total of 51Mbp in 69098 distinct contigs of sequences not present in GRCh38. The contigs were then aligned to the comprehensive blast database, a recently published catalogue of Icelandic novel sequences, the unmappable sequences of the Pan-African genome, as well as the chimpanzee genome, revealing a great diversity of shared sequences. Additionally, the unmappable sequences were searched for repeats and repeat elements: revealing a majority (58%) of repetitive sequence, consisting mainly of satellites (19%) and simple repeats (26%). Clustering all 1000 individuals based on their novel sequences resulted two clusters of similar size. One cluster is characterized by a higher degree of shared novel sequences, while the other was found to be diverse: as if sampled from a founder population, as well as a large, and diverse minority population. Finally, by aligning publicly available low coverage whole genome sequencing data from 23 Viking age individuals to our modern Swedish novel sequence catalogue, we show that genome sequences from Viking age individuals are enriched in novel sequences from modern day Swedes.

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P18.55C
Identifying signatures of positive selection in the Lithuanian population from high-density SNP data

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A characterization of the adaptive history in particular populations is of great importance as it provides knowledge of the genes that have been targeted by positive natural selection at a local geographical scale. We carried out genome-wide scans for different signatures of positive selection in a total of 399 individuals from the Lithuanian population.

We analysed Illumina 770 K HumanOmniExpress-12 v1.1 and Infinium OmniExpress-24 array data from Lithuania and combined it with that of the CEU, FIN and YRI populations from the 1000G project. Signatures of positive selection were then investigated using three statistics: Tajima's D, F_{ST} and XP-EHH.

We detected a total of 42 candidate regions with signatures of recent selection in the Lithuanian population. Few new strong signals of selection comprising several interesting genes were identified when comparing LT to the YRI population. One of such signals was found in chromosome 3, which comprises the *COL6A5* and *COL6A6* genes encoding for the collagen type VI alpha 5 and alpha 6 chains, respectively. Moreover, a non-synonymous variant in *COL6A5* (rs12488457) with a CADD value of 23.2 was found among the top XP-EHH and F_{ST} outliers along the region.

As expected, other candidate regions for positive selection identified in the Lithuanian population were related with pigmentation (*SLC24A5, TYRP1*), the immune

response (*BRD2, HLA-DOA, IL26, IL22*) and other traits and were partly shared with other European populations.

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P18.56D

Time-related differences in pregnancy duration distributions of relatives and its effect on heritability estimates

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Background: In determining the contribution of genetic factors to variation in pregnancy duration, it has been suggested that the core assumption underlying heritability estimation, equal environmental conditions in which relatives grow, might not be met when analyzing relatives coming from different generations.

Aim: Exploration of time-related environmental distributional differences, their detection and significance for the assessment of similarity in pregnancy duration between relatives.

Methods: 454435 parent-child, 2247065 full-siblings, 405116 maternal- and 469995 paternal- half-siblings pairs were retrieved from the Swedish Birth Register. The presence of time-related environmental changes was determined by analyzing the shape of pregnancy duration distribution over the years 1973 - 2012. The effect on the correlation estimate was evaluated by analyzing variation in the magnitude of correlation estimates between the relatives born in periods of time with differently shaped pregnancy duration distributions, and relatives with different age-gaps in between.

Results: Over the years, the distribution of pregnancy duration is left-shifting. Distributional differences were observed among relatives born in largely spaced periods of time. Decreasing correlation estimates together with increasing age-gap was observed among all siblings pairs. On the other hand, among full-siblings born within 2 years of one another, the correlation estimate increases significantly over the years, with some decrease in the mid-1990s.

Conclusion: The assumption of equal environmental conditions, in the case of pregnancy duration, is difficult to be met due to broad palette of environmental factors affecting the outcome. Caution when interpreting the heritability estimates must be taken.

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P18.58B

Colonexpress browser: An interactive web application for exploring gene and transcript expression in normal colon tissue

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Introduction: Understanding the genetic regulation of healthy tissues may contribute to explain the missing heritability of complex diseases. In this study, we conducted quantitative trait locus (QTL) analyses to associate genetic variation to gene and transcript expression in normal colon tissue and developed an interactive web application to explore in deep the results.

Materials and Methods: Colon biopsies were obtained from 191 healthy volunteers at colonoscopy. Normalized gene and transcript expression, obtained from RNA-Seq, and ~5 million Single Nucleotide Polymorphisms were analyzed using the R packages MatrixEQTL and sQTLSeekeR for expression and transcript ratio QTL analyses, respectively. Shiny was used to develop the application. We envisage enlarging the analyzed data set to nearly 500 samples during the following months.

Results: The web app Colonexpress browser allows to explore colon gene and transcript expression levels and download custom plots, such as scatter plots colored by variables of interest or annotated heatmaps where the splicing diversity can be observed. Lowly expressed transcripts can be grouped at a desired threshold. Also, the application allows to browse the QTL catalog and downloading tables

with statistics. It will be available at https://shiny.snpstats.net/colonexpress_browser.

Conclusion: Colonexpress browser allows the interactive visualization of a useful data resource for conducting further functional studies, which may help understanding association studies for diseases affecting colon tissue.

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P18.59C
Towards estimating the incidence of rare diseases in a paediatric population, born in Ireland in the year 2000

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Introduction: The EU recognises Rare Diseases as chronic, life threatening illnesses. National epidemiological studies of RD prevalence are uncommon. This study aims to derive the incidence of paediatric RD and its mortality case number for children born in the year 2000.

Materials and Methods: Cases were identified using electronic/manual records from: National Paediatric Mortality Registry office; Clinical, Cytogenetics and Molecular genetics database; Radiology and the Hospital In-Patient Enquiry system (HIPE). In addition, a detailed analysis of 10years national death registration information for RDs from 2006-2016 was undertaken along with a 2year study (2015-2016) of inpatient RD deaths.

Results**:** There were 54,789 livebirths in 2000. Clinical, Cytogenetic and Molecular Genetics identified 603, 121 and 77 cases of RDs respectively. HIPE searches (two major centres) identified 370 and 702 additional cases of RD. Mortality data (2006-2016) revealed 65.7% of 105 deaths from the 2000 cohort had a RD. Of all deaths on the Register (2006-2016), (n=4044) aged 0-14, 58.56% (n=2368) had a RD diagnosis. Of the total hospital days used by this cohort (n=5566.5) 84%(n=4668.5) of the total day usage and 77% (3137/4059) of ICU days used were by RD patients.

Conclusions: This study has identified > 1,800 RD patients presenting by age 17 giving a minimum incidence of 3.3% for paediatric RDs. 65.7% of paediatric mortality cases for the year 2000 cohort and 58.6% of all cases from 2006-2016 had a RD. It confirms that the use of acute hospital services by RD patients far exceeds that expected by their numbers.

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P18.60D
Estimating global point prevalence of rare diseases: analysis of the Orphanet database

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Introduction: Rare diseases (RDs) are an emerging global public health priority. These chronic, degenerative and life-threatening diseases are scarce and diverse, resulting in a lack of knowledge and expertise. Orphanet (www.orpha.net) is a publicly accessible RD knowledge base containing curated epidemiological indicators. Accurate epidemiological information about RDs as a group is necessary to inform public policy.

Methods: Univariate analysis of Orphanet data (www.orphadata.org). Global point prevalence of RDs was determined from the 'Orphanet Epidemiological File' by selecting RDs described by 'point prevalence' from predefined geographic regions for analysis, and summing point prevalences.

Results: Point prevalence is the appropriate epidemiological indicator for 85.9% (n=5304) of RDs and annual incidence for 14.1% of RDs. 71.7% (n=4425) of RDs are genetic (known/suspected familial; inherited or *de novo* single gene disorders; mitochondrial diseases; and chromosomal rearrangements). 11.9% of RDs are exclusively adult-onset. The minimum cumulative point prevalence of RDs was calculated of 3.5-5.9%, or 263 to 446 million persons affected worldwide by RDs at any point in time. While 84.5% of the 3585 RDs analysed had a point prevalence of < 1/1,000,000, 77.3-80.7% of the population burden of RDs was attributable to 149 (4.2%) diseases with prevalence of 1-5 per 10,000; and >95% of the population burden of RDs was attributable to 390 diseases.

Conclusion: Our conservative estimate of 3.5% to 5.9% is the first evidence-based estimate of the population prevalence of RDs. Future RD registry research and the

implementation of RD codification in healthcare systems will further refine these estimates.

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P18.61A
Rare variant association tests in presence of heterogeneity between cases

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The wide availability of Next Generation Sequence data opens new opportunities to discover rare variants associated to rare or common diseases. Rare variants association tests have been proposed specifically to this end. To obtain a higher statistical power, they test association between a whole genomic region (usually encompassing one gene) and the disease. These tests can be broadly classified in two categories: burden tests (CAST, WSS) and variance tests (SKAT).

However, even these tests may have an unsatisfactory power, due to the limited size of case samples and to the large number of genes to be tested when an agnostic approach is used. In this context, incorporating information on clinical heterogeneity among cases, e.g. differences in disease presentation, severity or age at onset, is an appealing way to build association tests with a higher sensitivity.

We propose extensions of both burden and variance tests to the situation where the cases are divided in such subgroups. The burden tests were extended by means of a multinomial logistic regression. A geometrical interpretation of the SKAT test for binary phenotypes was used to construct a natural extension of this test to our setting. The power of these tests was investigated under various simulation scenarios. They were also applied on some real data examples.

An efficient implementation of the proposed tests has been made available in an R package, Ravages, which is deposited on github (https://github.com/genostats/Ravages).

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P18.62B
Exploring the overlap between rheumatoid arthritis susceptibility loci and long non-coding RNA annotations

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Introduction: The interrogation of rheumatoid arthritis (RA) susceptibility loci has focussed on disruption of protein-coding genes and enhancers, however these features are unlikely to mediate the association with disease risk at all identified loci. Long non-coding RNA (lncRNA) have previously been demonstrated to be enriched within genome wide association study (GWAS) loci, however the significance of this enrichment, its relevance, and its generalisability to individual traits is unknown.

Materials and Methods: Using fgwas, the ability of individual annotations to improve a probabilistic model of disease susceptibility was tested. Publicly available lncRNA annotations, including lncRBase, lncipedia and the miTranscriptome assembly, were incorporated, in addition to cell type specific chromatin states annotations.

Results: Using fgwas it is possible to reproduce a previously reported enrichment of enhancers active in primary T helper cells amongst RA GWAS loci ($\log_2$ enrichment 2.96, 95% confidence interval [2.01, 3.69]), however estimates for enrichment of lncRNA are statistically insignificant. Interestingly, the miTranscriptome assembly, previously used to demonstrate an enrichment of lncRNA amongst GWAS loci, is demonstrated to be depleted amongst RA loci, using fgwas ($\log_2$ enrichment -0.80, 95% confidence interval [-1.70, -0.08]). The enrichment estimate associated with alternative databases of lncRNA seems to vary based on the protein-coding potential threshold applied to discriminate against protein-coding transcripts and appears to be independent of chromatin state annotations.

Conclusions: Whilst at specific GWAS loci, long non-coding RNA may represent interesting candidates for functional interrogation we found no evidence to support an enrichment of long non-coding RNA amongst RA susceptibility loci.

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P18.63C
Distribution of Runs Of Homozygosity (ROHs) along the human genome is shaped by recombination and purifying selection

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Whereas the higher fraction of the human genome, covered by Runs Of Homozygosity (ROHs), was associated with decreased height, decreased educational attainment and other phenotypes, the mapping position of the contributing ROHs was not assessed in details. Here, analysing genome-wide distribution of ROHs in offsprings of 100 consanguineous families we observed a non uniform distribution. Using multiple linear model we demonstrated that this variation could be explained by (i) recombination level, i.e. the higher the recombination in a given region, the less the number of ROHs; and (ii) selection, i.e. the higher the pLI score (which estimates loss of function intolerance) of the embedded genes the less the number of ROHs. The observed selection component might be explained by elimination of carriers of homozygous recessive loss of function mutations in genes with high pLI. To examine the selection component further we stratified ROHs by age and length following a previously suggested classification: class A (old and short: < 0.6 Mb), class B (intermediate in age and length: 0.6 - 1.6 Mb) and class C (young and long: > 1.6 Mb) and rerun our analyses. We observed that while the distribution of all three classes is shaped by recombination, only the class C ROHs showed a strong negative relationship with pLI, suggesting that younger ROHs are under stronger selection. Altogether our results provide additional metrics (properties of affected genes, ROH classes), which should be taken into account inferring deleterious effect of ROHs in GWAS and genetic medicine.

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P18.64D

Secondary findings - should we focus only on those relevant for individual health, or also on those with implications for further reproduction in the family?

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The increasing implementation of exome sequencing (ES) leads to growing number of secondary findings (SF). Following the recommendations of the American College of Medical Genetics and Genomics (ACMG) we started to build a pilot algorithm for SF management based on ES data from families analysed in research setting due to intellectual disability in their children. The ACMG recommendations focus on genes influencing health risks for individuals themselves. However, most families are tested as trios, and the couples are often young and planning to have another child. The data contain information on parental carrier status for severe hereditary disorders, and SF may also be extended to variants with implications for future children and reproductive choices. Therefore, our pilot SF algorithm combines evaluation of ACMG-recommended genes and analysis of parental carrier status for risk variants in recessive and X-linked genes. Until now, 260 individuals from 77 families have been analysed. The data show that at least 2.3 % of individuals carry variants in ACMG-recommended genes reported in ClinVar as pathogenic/likely pathogenic (e.g. BRCA2, RET), and couples may exist where both partners carry variants causing a recessive disorder (e.g. VPS13B). We propose discussion on the extension of SF to variants associated with risks for future reproduction. The next stage of the algorithm will be to explore the inclusion of this extension into informed consent and genetic counselling. Discrepancies in variant classification, need of expert analyses of individual genes and high time demand represent further obstacles in this effort. Supported by 17-29423A and 00064203.

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P18.65A

Admixture mapping study of sepsis in an African-admixed population from southwestern Europe

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Introduction: Sepsis is a severe systemic inflammatory response to an infection that is accompanied by organ disfunction. Although the ancestral background is an important factor for sepsis susceptibility and prevalence, no study has leveraged the genetic footprint of a recent admixture in the population for the identification of loci involved in sepsis susceptibility. Here we describe the results of the first admixture mapping study in sepsis.

Material and Methods: Local ancestry blocks obtained from genome-wide data from 113,414 SNPs genotyped in 343 sepsis cases and 410 unrelated controls, ascertained for grandparental origin in the Canary Islands (Spain), were used for the analysis. Significance was declared based on the number of ancestry blocks ($p<1.82e-4$). Fine mapping analysis were assessed with logistic and conditional regressions on HRC-imputed data.

Results and Conclusions: A significant hit was identified in a region spanning 1.2 Mb of chromosome 8q23.1 for the European ancestry supported by 114 SNPs (lowest $p=1.37e-4$; OR=0.51; 95%CI=0.40-0.65). 8q23.1 contains *MFHAS1* gene, which encodes a regulator of the Toll-like receptor 2 and 4 signaling pathways. MFHAS1 has been proposed as a biomarker for sepsis and genetic variants of the gene have been associated with immune diseases. Our results revealed a new genetic locus with plausible implications in sepsis susceptibility.

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P18.66B
Transcriptome-wide causal analyses reveals sex-specific biomarkers for complex human traits

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The prevalence of many diseases differs between men and women. Identification of sex-specific GWAS loci can help understanding the mechanisms underlying such sex differences. To address this challenge, we applied a transcriptome-wide summary statistics-based Mendelian Randomization approach (TWMR) to waist hip ratio (WHR) combining whole-blood eQTLs and sex-specific GWAS summary statistics from UKBiobank. Our results recapitulated the marked sexual dimorphism known for WHR: in the sex-combined analysis, we found 203 genes causally associated with WHR; of note, 38 and 26 genes were causally associated to WHR in women or men only, respectively (top genes: *ENTPD6*, P_{TWMR}=7.9E-11 in men; *CALCRL*, P_{TWMR}=4.6E-12 in women). Consistently, 16 of these 64 genes showed significant sex-difference (*P-diff*<0.05/203=2.5E-04). Conducting TWMR separately in men and women not only improves power to identify sex-specific associations, but also allows testing for sex differences. As might be expected, we did not observe the same power improvement when we applied TWMR to traits not showing sexual dimorphism, such as educational attainment (EA). Out of the 49 genes causally associated with EA, only 4 and 5 were specific to women and men, respectively. Although previous data indicate that eQTL effects are not strongly influenced by sex, it will be crucial to perform the same analyses with sex-specific eQTL data generated from larger data sets to dissect sex-specific effects appearing up- *vs*down-stream of gene regulation. Our findings demonstrate the importance of investigating sex differences, which may lead to a better understanding of disease mechanisms facilitating treatment options and precision medicine.

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P18.68D
High-dimensional analysis of SNP array data reveals major subdivisions in the Canary Islands (Spain) population

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Introduction: Based on SNP array data, model-based ancestry estimations, and principal component (PC) analysis, we have recently conducted a detailed genomic analysis of the current inhabitants of the Canary Islanders (Spain), revealing the existence of a significant African influence and population isolation. Here we have reassessed their genomic diversity and structure based on alternative high-dimensional analysis techniques.

Materials and Methods: A total of 117K variants from 618 unrelated subjects from the seven main islands of the archipelago ascertained based on grandparental birthplace were compared. Topological data analysis (TDA) was conducted using umap v0.2.0 R library both based on raw genotyping data and 2-to-20 PCs derived with PLINK v1.9.

Results: Three main population-aggregated clusters were identified: two corresponding to the smallest islands of the archipelago (El Hierro and La Gomera), and a Y-shaped cluster aggregating the rest of Canary Islanders. In addition, TDA based on six PCs showed intriguing subdivisions within La Gomera, the population showing the largest North African influences described so far for a southwestern European population.

Conclusions: High dimensional data analysis suggests that extreme isolation in the populations from El Hierro and La Gomera is a major source of genetic sub-structure in Canary Islanders.

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P18.69A

The association between telomere length and cardio-metabolic components: a Mendelian randomization study

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Introduction: Telomeres are the tips of chromosomes and are composed of proteins and several thousand copies of a hexamer repeat sequence (TTAGGG)n. Telomere length (TL) is shortened progressively with age. Many studies revealed that age, sex, ethnicity, smoking, obesity, lack of exercise could result in shorter telomere length. However, these factors associated with TL are also contributed to cardio-metabolic components/diseases. Several genetic loci associated with telomere length have been identified. The study was aimed to assess whether telomere length causally affects cardio-metabolic components using Mendelian randomization design.

Methods: A total of 2543 adults were recruited from the Matsu community-based integrated health screening project. Five SNPs (*TERC* rs12696304, rs16847897, *TERT* rs2736100, *FTO* rs9930506, rs9939609) associated with telomere length were used as instrument variables to estimate the causal effect of telomere length on cardio-metabolic components by Mendelian randomization analysis using two-stage least-square instrumental variable (IV) regression.

Results: We found that both *FTO* rs9930506 and rs9939609 SNPs were significantly associated with telomere length ($p<0.05$), but not the other three telomere length related SNPs. The results of linear regression showed that telomere length was not significantly associated with waist circumference, body mass index, blood pressures, fasting glucose, lipid levels (all with $p>0.05$). Mendelian randomization using two-stage least-square IV regression did not show that telomere length instrumented by the two *FTO* SNPs, were associated with any cardio-metabolic components ($p>0.05$).

Conclusion: Our study could not confirm the previous knowledge of the causal effects of telomere length on cardio-metabolic components. Grant No: MOST 106-2314-B-010-020-MY3

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P18.70B

A large spectrum of variants identified in moderate thrombocytopenic French blood donors: results of the ABC study

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Moderate thrombocytopenia (less than 150G/l) is a reason for donors deferral for blood donation in France. We aimed at exploring potential genetic variations responsible for these defects and to define their prevalence in a blood donor population from 3 French regions: Alsace, Centre-Atlantique and Bretagne. We collected a saliva sample from 448 donors with platelet counts <150 G/l on two consecutive blood donations. Following DNA extraction the coding sequences and intron/exon junctions of 17 candidate genes were amplified by PCR Ampliseq™ and sequenced on a Ion Proton©. Annotation of the variants was performed (SeqNext© software). The pathogenicity of the variants was assessed using data from the literature and bioinformatics prediction tools. Most donors (59%) were aware of their low platelet count but only 17% knew a family history. 84 heterozygous variants of interest spread over 12 genes. 23 variants are deleterious, the rest of the variants being of unknown significance 59 variants were found on gene already studied in patients with inherited thrombocytopenia (8 *GPIBA*, 2 *GPIBB*, 3 *WF*, 9 *MYH9*, 11 *TUBB1*, 9 *ACTN1*, 13 *ITGA2B* and 5 *FLNA*) with a surprisingly high frequency of ITGA2B and TUBB1 variants. The remaining 24 variants where identified on genes suspected to be responsible for thrombocytopenia in human or already studied in mouse models (7 *TUBA8*, 8 *ITGB3*, 5 *ITGA2*, 2 *RASGRP2* and 2 *TUBA4A*). These results indicate that at least 5% of healthy donors with moderate thrombocytopenia carry a constitutional anomaly in a gene responsible for this platelet count defect.

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P18.71C

Topological data analysis of SNP array data exposes the genetic differentiation between Iberians and Canary Islanders

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Introduction: Genetic structure of populations often necessitates multidimensionality reduction, typically assessed with principal component (PC) analysis (PCA). However, such procedure most commonly focuses on a few main dimensions limiting the possibilities to excavate fine-grained strata. Here we used topological data analysis (TDA) to embed high-dimensionality of SNP array data to explore the degree of genetic differentiation between Canary Islanders and the Iberian Peninsula population (IBS).

Materials and Methods: We used data from 46 Canary Islanders along with 740 unrelated subjects from IBS and 23 North Africans (NAF) for reference. PCA and TDA were assessed on quality-controlled data using the PLINK v1.9 and umap v0.2.0 library for R.

Results: By leveraging the information from as few as 136K independent SNPs embedded within a 2-to-50 PC space, we found highly distinctive patterns of differentiation between Canary Islanders and the IBS populations despite their shared Spanish ancestry. Strikingly, most Canary Islanders clustered closer to NAF, although a few individuals clustered with IBS.

Conclusions: TDA provides an optimal alternative to reveal previously unrecognized fine structure separating IBS from Canary Islanders, compatible with genetic drift and differential admixtures. Co-clustering of Canary Islanders both with NAF and IBS supports wide interindividual variation in ancestries, reflective of their recent history.

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P18.72D

In-silico analysis of Barrett's esophagus and esophageal adenocarcinoma reveals an association between the genetic signal and gene expression regulation in esophagus tissues

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Introduction: Barrett's esophagus (BE) is a premalignant precursor of Esophageal adenocarcinoma (EA) which is one of the leading causes of cancer deaths. Both conditions are complex traits and we aimed at dissecting their polygenic structure in association with tissue specific gene expression regulation.

Materials and Methods: We estimated the SNP-heritability on the liability scale and genetic correlation by applying LD score regression analysis to the largest meta-analysis results (6,167 BE cases, 4,112 EA cases, 17,159 controls). We performed Polygenic Risk Score (PRS) modeling. We analysed gene expression alterations by means of Transcriptome Wide Association Study (TWAS) and FUMA platform. We modeled the gene expression PRS (GE-PRS) based on the PrediXcan- and MetaXcan-imputed gene expression profiles.

Results: SNP-heritability (h2) for BE was h2=0.20, h2=0.13 for EA, and h2=0.15 for the combined cohort (BE and EA). The genetic correlation between BE and EA was r_g=0.85. We identified a strong association of PRS comparing BE against controls ($p=3.7*10^{-5}$), EA against controls ($p=3.5*10^{-7}$), and the combined cohort (BE and EA) against controls ($p=5*10^{-13}$), but no association comparing BE against EA (p=0.09). Esophagus tissues were prioritized as the most significant by FUMA, and the TWAS uncovered particular genes among these tissues (e.g., *BTN3A2*) whose expression was significantly altered ($p=3*10^{-7}$). Finally, the GE-PRS model in the esophagus mucosa tissue was significantly associated with the case/control status ($p=1.2*10^{-8}$).

Conclusion: Our work dissected a strong polygenic component of Barrett's esophagus and Esophageal adenocarcinoma, we identified potential candidate genes and established gene expression alterations in disease-specific tissues.

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P18.73A

Cross-phenotype transcriptome-wide association study reveals shared susceptibility genes between schizophrenia and inflammatory bowel disease in gut-brain axis related tissues

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Introduction: Genome-Wide Association Studies (GWAS) have identified hundreds of genetic loci both for schizophrenia (SCZ) and inflammatory bowel disease (IBD). Noteworthy, both increased prevalence and genetic correlation have been reported for SCZ and IBD, which suggests potential shared molecular mechanisms between these traits.

Materials and Methods: We performed a cross-phenotype transcriptome-wide association study (TWAS) using the largest available summary statistics for SCZ (36.989 cases, 113.075 controls) and IBD (18.431 Crohn's disease cases, 14.191 ulcerative colitis cases, and 33.658

controls). We imputed the genetically regulated component of gene expression by integrating GWAS signals with tissue-specific Expression Quantitative Trait Loci (eQTL) in 48 tissues. We used models trained on data from the Genotype-Tissue Expression Project (GTEx).

Results: The combined evaluation of SCZ and IBD TWAS results revealed a significant enrichment of associated genes overlapping between SCZ and IBD in different tissues. Interestingly, colon and brain were among the top significant tissues. Genes from the major histocompatibility complex (MHC) are also among the most strongly TWAS-associated genes, which is in line with corresponding genetic signals in SCZ and IBD.

Conclusion: Our TWAS results suggest that the genetic correlation between SCZ and IBD can be associated with gene expression alteration in tissues involved in the regulation of the gut-brain axis and that MHC genes may play a pivotal role. In order to further investigate this hypothesis, we intend to perform cross-phenotype TWAS including other psychiatric and immune-related traits showing genetic correlation.

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P18.74B

Phenome-wide study of *TTR* non-coding variation in UK Biobank participants: new knowledge regarding the pathogenesis of hereditary and senile systemic amyloidosis

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Introduction: *TTR* coding mutations are responsible for a hereditary form of amyloidosis (ATTR), which presents a complex genotype-phenotype correlation including a wide range of clinical signs (i.e., peripheral and autonomic neuropathy, cardiomyopathy, nephropathy, gastrointestinal or ocular impairment). Additionally, a form of senile systemic amyloidosis (SSA) is due to the misfolding of the protein product of the same gene. Non-coding variation in *TTR* gene appears to have a key role in affecting the clinical phenotype of carriers of coding mutation and increasing the risk of SSA in non-carriers. To understand the role of *TTR* gene non-coding variation, we conducted a phenome-wide association study in the UK Biobank.

Materials and Methods: We investigated 18,823 variants located in *TTR* gene and its surrounding genomic regions (GRCh37 chromosome 18: 27171000–31171500) in 361,194 participants from the UK Biobank with respect to more than 4,000 traits, which include a wide range of physiological and pathological conditions.

Results: *TTR* non-coding variants were significantly associated with multiple phenotypic traits after multiple testing correction (false discovery rate q-value < 0.05). Among these significant results, we observed several associations related to known signs of TTR amyloidosis: renal failure (rs150146618, $p=1.33x10^{-6}$); disorders related to central nervous system (rs8091089, $p=1.24x10^{-6}$); and cardiac failure (rs145777295, $p=2.52 \times 10^{-6}$). Additionally, a sex-stratified analysis showed significant differences between females and males in the effects of *TTR* non-coding variants.

Conclusions: The study provides novel insights regarding the molecular mechanisms involved in ATTR and SSA, demonstrating the effect of *TTR* non-coding variation on human phenome.

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P18.75C

Combined effect of genetic predisposition, obesity, and favorable lifestyle on risk of type 2 diabetes

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Objective: To study whether genetic risk of type 2 diabetes (T2D) can be attenuated by adhering to a favorable lifestyle and retaining normal body weight.

Methods: We applied Prentice-weighted Cox regression models to a case-cohort sample of 9,556 men and women from the Danish prospective Diet, Cancer and Health cohort. 49.5% of the participants developed T2D during an average 12 years of follow-up. A favorable lifestyle was defined as having three of the following healthy lifestyle factors: no current smoking, moderate alcohol consumption, regular physical activity, and healthy diet. An unfavorable lifestyle was defined as no or only one healthy lifestyle factor while the remaining participants were defined as having an intermediate lifestyle. Genetic risk was assessed by a genetic risk score (GRS) comprising 213 genetic loci robustly associated with T2D. The GRS was stratified into low (lowest 25%), intermediate (middle 50%) and high risk (top 25%) groups.

Results: Adherence to a favorable lifestyle and normal-weight decreased T2D risk independent of genetic predisposition (P>0.05 for GRS-lifestyle and GRS-obesity interaction). Obesity (BMI ≥30 kg/m^2) increased T2D-risk by 5.8-fold (95% CI: 5.2-6.6) compared to non-obese individuals, while the independent effects of high (vs. low) genetic risk and unfavorable (vs. favorable) lifestyle were relatively modest (HR=1.8, 95% CI 1.6-2.0; and HR=1.2, 95% CI 1.1-1.3, respectively).

Conclusions: Individuals with poor lifestyle and obesity are at greater risk of incident T2D regardless of their genetic risk.

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P18.76D
The relationship between genetically raised bilirubin and respiratory cancer: a UK Biobank study

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Introduction: Genotypes causing mild hyperbilirubinaemia (OMIM:#143500) are common in human populations ranging from 10% (Europe and East Asia) to 25% (equatorial Africa). Basic studies suggest moderately raised bilirubin may be beneficial and protect respiratory systems against oxidative stress from infection, smoke and pollution. Here we report the results of the first large-scale Mendelian randomisation examining the causal relationship between serum bilirubin and respiratory cancer.

Materials and Methods: This research has been conducted using unrelated participants of European ancestry from the UK Biobank Resource. Homozygosity for the minor T allele of rs887829 in the UDP-glucuronosyltransferase 1-1 gene is associated with serum bilirubin levels 8-10 μmol/l (80-100%) higher than those without this genotype. Using multivariable Poisson regression, we analysed the relationship between rs887829 and the incidence of respiratory and intrathoracic cancers derived from national registers. All analyses were stratified by smoking status with heavy smoking defined as ≥20 cigarettes per day.

Results: 323,702 participants (median follow-up 5.5 years) and 918 cancer events were included in the analyses. Overall homozygosity for the T allele of rs887829 (n=31,794) was associated with a 22% lower incidence of respiratory cancer (95%CI:0-48%; p-value=0.047) compared with other genotypes. Stratified analysis revealed this effect was driven by heavy smokers with a 59% lower incident rate (95%CI:38-76%; p-value=0.0018).

Conclusion: Genetically raised bilirubin could help protect people exposed to high levels of oxidants against respiratory cancers. Serum bilirubin may therefore represent a therapeutic target as well as a low-cost biomarker for disease risk stratification. Wellcome Trust funded:209207/Z/17/Z

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P18.77A
Genetic contribution of visceral adiposity and its relation to cardiovascular and metabolic disease

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The genetic contribution to, and the disease-related effects of visceral adipose tissue (VAT) are largely unexplored due to the requirements of advanced imaging technologies to accurately measure VAT. We therefore developed non-linear prediction models of VAT mass using 4,198

individuals of white British ancestry from UK Biobank (UKBB). VAT mass was then predicted in 325,153 UKBB participants, aged 40-73 years. We performed a genome-wide association (GWA) study for predicted VAT mass (VAT^) and estimated the association between VAT^ and hypertension, heart attack/angina, type 2 diabetes, and hyperlipidemia. Causal effects on disease risk were assessed by Mendelian randomisation. The GWA study identified 209 independent genetic associations for VAT^, representing 102 novel loci. Functional analyses showed a significant difference, both in expression and promoter activity between the alleles of rs1799993, located in the *HMBS* promoter. VAT^ was associated with increased risk of disease with a more pronounced effect in females compared to males ($p<0.0001$). The largest observed effect was found for type 2 diabetes. Furthermore, a pronounced non-linear effect of VAT^ on disease risk was uncovered for all four diseases ($p<0.0001$). Finally, Mendelian randomisation showed VAT^ to be a casual risk factor and a remarkably high causal OR=7.34 (95% CI 4.48-12.0) was found for type 2 diabetes in females. Our findings substantially bolster the role of visceral adiposity as a bona fide predictor.

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P18.78B
Exome sequencing identifies novel rare variant association signals in the Cooperative Health Research in South Tyrol (CHRIS) study

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Introduction: Thus far, only few rare variant association signals have been identified. Given their expected larger impact on complex traits, it is valuable to study their association in different contexts (e.g., homogeneous environmental conditions) and not only in large biobanks.

Materials and Methods: Whole exome sequencing (WES) association analyses were performed for association with 56 biochemical traits in 3840 individuals from the population-based CHRIS study using the xGen Exome Research Panel (v1.0) and processed using the Genome Analysis Toolkit (GATK v3.7) pipeline. After QC, 3422 samples remained, with a mean target coverage of 68.4X. WES variants were validated against Infinium Omni2.5Exome BeadChip genotype data, with which there was concordance of 0.998 across overlapping variants. We performed single-variant association analysis using EMMAX to account for family structure, and gene-based association analysis using SKAT, grouping variants masked as either "LoF" or "high-moderate impact". We used VEP/LOFTEE for annotation. For both approaches, analyses were repeated conditioning on all previously known variant-trait associations collated from the EBI GWAS catalog, to ensure novelty of identified associations.

Results: In total, 825,158 rare variants (MAF<0.5%) were identified across all samples. The single variant association analysis identified 60 novel variant-trait associations (P<2e-7), which clustered to 22 loci-trait associations. Gene-based association analysis yielded 27 significant gene-trait associations (P<3.3e-6), of which 5 were completely novel, and 5 were novel gene-level signals near known single-variant associations.

Conclusion: In the age of large Biobanks, relatively small but well characterized population-based studies remain important for elucidating the genetic architecture of quantitative traits.

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P18.79C
Cataloging genetic variation in Canary Islanders by means of whole exome and genome sequencing

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Introduction: The genetic uniqueness of the current Canary Islands population (Spain) results from isolation, local adaptation, and recent admixture events mainly involving individuals of Europe, Northwest-Africa and a minor contribution from Sub-Saharan populations. These conditions and the limited characterization of the genetic pool of the African populations justify the necessity of building a reference dataset to be instrumental for Precision Medicine in the archipelago. Here we provide details of the design and preliminary results of this catalog.

Materials and Methods: A target sample of 1000 controls is being characterized by whole-exome sequencing (WES) using Nextera-DNA Exome kit (Illumina) on HiSeq4000 and Axiom Genome-Wide Human CEU 1 array technologies. In addition, whole-genomes of a subset of the donors are being obtained with two complementary sequencing technologies (Illumina and Oxford Nanopore Technologies).

Results: Array-based-genotypes and whole-exome sequences of 317 controls have been analysed so far. A total of 816,829 variants have been identified by WES, of which 55% and 47% are not described in ExAC nor in gnomAD, respectively, while 59% of them are classified as missense or nonsense.

Conclusions: These results evidence the necessity of a detailed catalog of genetic variation in the Canary Islands to improve the identification of disease genes and facilitate the identification of pathogenic variants.

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P18.80D
A whole-genome scan of natural selection in Canary Islanders

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Introduction: The genetic uniqueness of the current Canary Islands (Spain) inhabitants results from the historical admixture with sources from Northwest Africa (NAF), Europe (EUR) and the sub-Saharan Africa (SSA). We used whole-genome sequencing (WGS) to identify genomic regions under selective processes.

Materials and Methods: WGS from Canary Islanders (n=46) were obtained and variant calling was assessed using BWA-GATK. WGS data from NAF (n=23), and SSA and EUR from the 1000 Genomes Project (1KGP), were used for comparisons. Selection signals were identified with distinct neutrality tests. Values in the extremes of the distributions were prioritized and used for enrichment analysis.

Results: A total of 14.4 million variants were identified in Canary Islanders, and about a fifth was not described in 1KGP. Neutrality tests identified a number of putative regions of selection. Attending to PBS, the largest values were located in the HLA, supporting different independent signals. Autoimmune, ophthalmologic, and oncologic diseases were among the top-enriched terms in the prioritized regions.

Conclusions: Our results support the existence of a genuine gene pool in the current Canary Islanders. Studies to evaluate the relationship between the signals of selection and the admixture sources are needed.

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P18.81A
Increasing the value of the Swedish 1000 whole-genome data resource

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The SweGen data resource is a collection of 1000 human whole genomes (Illumina 30x coverage), which represents the genetic variation in the Swedish population. Since its first release in 2016, the SweGen variant data has become a widely used resource with about 200 monthly visits to the website both from Sweden and abroad (swefreq.nbis.se). During the course of the project it has become apparent that

the SweGen data can serve many more purposes than just providing information about SNPs and small indel variants in the Swedish population. We have therefore initiated a number of follow-up projects to increase the value of the resource. Examples of ongoing activities are: i) detection of complex structural variation, ii) analysis of genetic variation in the MHC region, iii) characterization of haplotypes of pharmacogenetic utility, iv) detection of somatic genomic aberrations, and v) utilization of SweGen data for improved SNP array imputation. In addition, we have constructed *de novo* high-quality reference genomes for one male and one female SweGen individual, based on multiple long-read sequencing technologies (PacBio, Oxford Nanopore, 10X Genomics and BioNano). These two assemblies are among the most complete individual human genomes to date, and surprisingly we detected over 10Mb of sequence in each individual that is missing from the current version of the human reference genome (GRCh38). Based on this missing sequence, we have constructed a modified version of the human reference, tailored for the Swedish population, that significantly improves the alignment and variant calling of the SweGen Illumina data.

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P18.82B
Analysing the joint effect of rare and common variants on human traits, using whole-genome sequencing data and a gene-based approach, increases the power to identify genetic effects

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Introduction: Despite the success of genome-wide association (GWA) studies, much of the genetic contribution to complex diseases and phenotypes remains unexplained. Two major limitations in GWA studies are the lack of power to test the effect of rare alleles and the use of genotyped or imputed SNPs.

Materials and Methods: Whole-genome sequencing (WGS) data and measurements of over 400 disease-related biomarkers were produced in 1,021 individuals from a cross-sectional Swedish cohort. We scored variants using CADD or Eigen scores. We then developed a pipe-line for exploring the combined effects of common and rare genetic variants on biomarker levels in a gene-wise manner.

Results: For coding variants, we identified 158 independent signals, of which 52 was in trans. Six of these were associated with the expression levels of several biomarkers. For regulatory regions, only cis-regulatory elements were analysed, and for as many as 191 biomarkers we identified at least one regulatory element that was associated with the expression of the biomarker.

Conclusions: We have shown that analysing the combined effect of rare and common variants in a gene-based approach increases the power to detect genetic effects, compared to a GWA study. Many of our associated signals contain multiple rare, and even population specific variants, which highlights the importance of more sophisticated methods for analysing WGS data in relation to human traits.

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P19
Genetic counselling - Services - Education

P19.02A
Genetic testing: panacea or Pandora's box? Questionnaire survey among Poles

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Aim: Analyse of Polish people approach to genetic testing. In addition, the doctors' knowledge on the same subject was checked Methods: Data collection was carried out by the use of a comprehensive questionnaire which was available to respondents on the Internet as well as in paper format. 2210 people were examined. The average age was 29 (between 18 to 80 years old). Group of doctors counting 204 people answered the same set of questions with few addition, the average age was 30 years old (between 27 to 57 years old).

Results: Many of respondents (37,7%) declared a genetic disease among their close relatives (family, friends). They mainly mentioned the Down Syndrome. Interestingly, 47.4% of them admitted to encounter stigma in everyday life. 9,9% of respondents was consulted by clinical geneticist. What is interesting 10,5%, who haven't seen the clinical geneticist, carried out a commercial genetic tests. Most test were done in search of the genetic basis of the diseases (63,6%). 88.9% of the respondents met with the term genetically determined cancer. 94.1% think that breast

cancer is included in that group of cancers, 62.6% consider ovarian cancer, and interestingly 43.4% also find a genetic basis for cervical cancer. Results for the same question in the medical group showed that 99.5% of physicians consider breast cancer, 93.3% ovarian cancer and 26.8% cervical cancer as partially hereditary cancers.

Conclusions: We should focus on developing opportunities to broaden the knowledge of both medical personnel and citizens.

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P19.03B

Genetic counselling for the Inuit indigenous population of Nunavut, Canada: an exercise in cultural competency

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The importance of providing culturally competent and culturally appropriate genetic counselling is recognized by the profession, as culture's role is crucial in molding an individual's conceptualization of health and illness. There is a dearth of literature on the provision of genetics services including genetic counselling for indigenous populations globally. Although exploratory studies have been published regarding the provision of genetics services for the indigenous populations of Australia, New Zealand, Philippines and the United States, few have described genetics services for the Canadian Indigenous populations (First Nations, Inuit and Métis peoples). Ottawa, Canada serves at the "local" genetics service for residents of Baffin Island, Nunavut, which has an Inuit population of approximately 15,000. We describe the Ottawa Baffin Nunavut Services (OBNS) program, as it relates to the provision of genetics services for Inuit persons, including travel coordination, lodging, traditional diet, Inuktitut interpretation services, and medical records coordination. We present a reflective review of cases illustrating the genetic counselling challenges, including building rapport, pedigree taking, patient isolation from their community, and cultural views of decision-making. We share personal stories of the humbling journey as genetic counsellors providing genetics services for Inuit persons from Nunavut and the lessons learned in cultural competency. Exploring these issues has never been more important, as Canada embarks on the *Silent Genomes Project*, which aims to improve the access to genomic technologies and research to Indigenous populations while ensuring Indigenous-led governance over biological samples and health data.

M. Cloutier: None.

P19.05D

EuroGEMS.org - the ESHG's new educational resources website for genetics professionals, students, schools and patients: international uptake and further development

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Introduction: Many highly-impressive, free, genetic/genomic educational online resources exist internationally but are often difficult to locate. The author's new, already-popular ESHG-supported website, ESHG Genetic Educational Materials and Sources (at www.EuroGEMS.org) provides a straightforward categorized guide with direct links to numerous excellent free online educational resources worldwide. These include professionally-created patient videos, sophisticated but entertaining animations and cutting-edge genomic databases.

Methods: Following discussion with colleagues, students and patients, >70 up-to-date, high-quality online worldwide sources were selected and categorized with concise explanations of each source's features. Included are multiple links to new, excellent but less well-known sites, plus ESHG pages and localized non-English-language initiatives. Professional-level web-hosting permits unlimited simultaneous visits.

Results: EuroGEMS.org was enthusiastically endorsed by the ESHG Board (ESHG-Milan-2018), and linked from the ESHG-Education web-page. To date, EuroGEMS.org has received 3318 views from 81 countries. The most frequently visited sub-section index pages are those for: "Genetics Professionals", then "Universities & Students", "Patients/Families", "Secondary Schools", "Primary Schools" and "Ethical, Legal & Social Implications". Radboud University's "Science Education Hub" is the most frequently visited linked resource. Students report that EuroGEMS.org is "wonderful for accessing genetics databases and looking up relevant genetic conditions."

Conclusions: An up-to-date informative guide to many of the best free worldwide educational resources has been created for the ESHG and accessed from 81 countries in just 8 months. The website's careful maintenance and development continues. Further suggestions for links are welcomed. The author is grateful to Education Committee Chair, Prof Han Brunner, for his welcome encouragement whilst creating this website.

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P19.06A
Online education to the masses enables transformative changes in healthcare

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Introduction: Genomics is revolutionising healthcare enabled by techniques such as whole genome sequencing, consequentially there is a pressing need to improve genomic literacy and data analysis skills in the healthcare workforce. In this study we demonstrate how development of the world's first free Massive Online Open Course (MOOC) in **Clinical Bioinformatics** has addressed these needs in healthcare professionals and patients and the public world-wide.

Methodology: The MOOC is hosted by FutureLearn, exploring the subject from its initial roots to clinical working practices, ethics and tools. The social constructivist approach embeds social discourse, enabling knowledge exchange between healthcare professionals, patients and carers. Social learning is encouraged using "follow", "like" and "bookmark" buttons to applaud specific comments.

Results: Within seven course runs 17, 000 learners joined the course, the largest numbers from the UK, Egypt, India and USA. Pre-course surveys showed that of those who stated their profession on enrolment, 31%, the largest group, identified as working in health and social care. The MOOC was most popular with participants in the aged 18-35, educated to at least degree level. Post-course evaluation showed 78% of 145 learners liked or strongly liked the online discussions and enjoyed interacting with other learners, 92% of 80 learners said they agreed or strongly agreed that the course had increased their understanding of clinical bioinformatics.

Conclusions: This course has provided knowledge and skills in bioinformatics and improved genomic literacy at a global scale, facilitating discussion between clinicians and the public regarding important areas such as data sharing. https://www.futurelearn.com/courses/bioinformatics

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P19.07B
Needs of the genetic counselling for familial cancer at a university hospital

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Introduction: Our genetic counselling departments were founded in 2007. Outpatient service specific to familial tumor started in 2013. In Japan, precision medicine will increase from April 2019 with the background of national policy. We consider that it is important to review the patients and their needs to prepare future outpatients.

Materials and Methods: We reviewed 100 cases of familial tumor from April 2009 to May 2018. Type of diseases, undergoing genetic test, results of the tests and their courses were examined retrospectively.

Results: We had 95 female patients and 5 male patients as proband. The number of cases increased after familial tumor service has been started. Type of diseases were hereditary breast ovary cancer (HBOC), Lynch syndrome, hereditary thyroid cancer and so on. 76 cases were suspected HBOC and 58/76 cases underwent genetic test. Of 58 cases, 56 cases had already developed cancer. The results of the test were positive for 12 cases. Positive genes were BRCA1 for 4 cases, BRCA2 for 4 cases and RET for 4 cases.

Conclusions: The genetic counselling for HBOC is the most required in our department. We need to expand collaboration system with not only breast cancer team but also gynecological cancer team. Also cooperation with ward nurses and other medical staffs would be needed to offer substantial support for patients.

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P19.08C
Exploring the experiences and support needs of non-carrier fathers of children with fragile X syndrome

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Clinical features of children with fragile X syndrome (FXS) include developmental delay or intellectual disability and behavioral, emotional and specific learning challenges. The diagnosis of FXS in a child often reveals the premutation carrier status of their mother. The genetic counseling process may therefore focus on the child and mother, without significant attention given to the information and support needs of the non-carrier father. This research sought to explore the experiences and support needs specific to non-carrier fathers of children diagnosed with FXS.

Eleven interviews were conducted with non-carrier fathers recruited through the Australian-based Genetics of Learning Disability (GOLD) Service and the Fragile X Association of Australia. Interviews lasted on average 78 minutes (range 61 – 102 minutes). Interviews were transcribed, deidentified, coded and analyzed by three coders using thematic analysis with an inductive approach.

Thematic saturation was reached. Four themes emerged: 1) Making Life Easier through Understanding – Yesterday and Today; 2) The Path to a New Normal – Today and Tomorrow; 3) Information and Support – Paper, Scissors, Rock; and 4) What Men Want.

Our study highlights some of the unmet support and information needs of non-carrier fathers of children with FXS. These include the need for practical and prognostic information, guidance in supporting their families, and flexible opportunities for accessing personal counseling and support. Our findings will inform the provision of more tailored support and information, not only for fathers of children with FXS, but for other neurocognitive conditions.

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P19.09D
Development of a short 6-item form of the Genetic Counselling Outcome Scale: The Genomics Outcome Scale

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Introduction: The Genetic Counselling Outcome Scale (GCOS-24) is a 24-item patient-reported outcome measure for evaluating genetic counselling and testing interventions. The aim in this study was to create a shorter form of GCOS-24.

Methods: Cognitive interviews explored interpretability of GCOS-24 items and items highly valued by the target population. The Graded Response Model (GRM) was used to examine item discrimination in an existing GCOS-24 dataset (n=395). Items with poor discriminative properties were then excluded and items capturing a similar concept were not selected together. Finally, quantitative and qualitative findings were combined to identify superior items. Rasch analysis was used to establish the optimal response scale.

Results: Ten cognitive interviews were conducted with individuals from families affected by genetic conditions, recruited through support groups. Qualitative analysis of interview transcripts identified twelve GCOS-24 items highly valued by participants. GRM item characteristic curves and item information curves were generated. Findings from both analyses were used to select ten items that were both highly valued and performed well. Finally, items were iteratively removed and permutated to establish optimal fit using the Rasch model. A six-item questionnaire with a five-point Likert Scale was created (The Genomics Outcome Scale (GOS)). Correlation between GCOS-24 and GOS is good (r=.838, 99% confidence), indicating that GOS maintains the ability of GCOS-24 to capture empowerment, whilst providing a less burdensome scale for respondents.

Conclusions: GOS will be less burdensome than GCOS-24 for patients and could be used where genetic testing is done outside the context of clinical genetics.

Unfunded study.

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P19.10A
The challenges of genetic counselling in consanguineous communities in North Israel

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The Genetics Institute of Emek Medical Center serves a population living in northern Israel, with significant representation of Israeli minorities, including Muslim and Christian Arabs. These populations are devoted to old traditions including consanguineous marriages, (presently 24.4%), leading to an increased risk of rare autosomal recessive disorders. Most of this population lives in small towns and villages that were founded by small number of individuals. Therefore, the diagnosis of founder mutations allows effective genetic counselling and occasionally genetic screening.

However, there are genetic mechanisms that make the counselling more challenging such as co- inheritance of more than one recessive condition in the same sib- ship. Each of these couples has 25% risk for an offspring affected by one condition and additional 25% risk for the other condition. Seven couples had 2 autosomal recessive conditions, diagnosed in different babies. For example, Congenital thrombocytopenia/Muscular Dystrophy, Wolman/ Meckel syndromes.

Counseling these couples often done following the birth of affected children, and is complex particularly after the second event. It is associated with marked emotional struggle of the parents, reduced self-confidence and a sense of control-loss, occasionally leading to mistrust of the counselling process. Family planning becomes more challenging, with pre-implantation genetic diagnosis become advantageous, a treatment associated with medical, technical and personal difficulties. Genetic counselling performed by experienced and aware professionals, might improve the outcome.

The new era of genomic technologies has dramatically improved our diagnostic abilities, and hopefully will allow us to detect those couples before the birth of their affected children.

O. Aboleil Zoubi: None. **S. Allon Shaliv:** None.

P19.11B
Genetic counseling from the perspective of modern ethical concepts: practical implications

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Introduction: Genetic counseling can be considered as the external image of Clinical Genetics. The way in which it is delivered is the basis for patients' and the health care system's evaluation of the efficiency of the field as a medical specialty. It is thus of utmost importance that genetic counseling professionals obtain an adequate preparation for its practice, in accordance with the complex character of genetic diseases.

Stern (Measuring Medical Professionalism, 2006) includes ethical and legal understanding as a foundation for medical professionalism. The ethics of genetic counseling is often reduced to the implementation of the requisite of nondirectiveness and of the elements of principialism: respect for autonomy, beneficence, non-maleficence and justice.

Aim and Methodology: This study aims to apply other bioethical concepts to the practice of genetic counseling. Principles derived from various modern philosophical and ethical modes of thought such as personalism, virtue ethics, narrative ethics and care ethics which have been applied to other medical fields or action, are herewith analyzed in the context of genetic counseling, specifically in prenatal diagnostics. Practical implications for counseling are deduced from this analysis.

Conclusion: An understanding of a wider range of bioethical concepts allows for a greater awareness of ethical requisites for proper genetic counseling. Given that this communication process frequently involves value-laden decisions on the part of patients and families, such an understanding can build better counselor-counseled relationships adequately facilitating well-informed, shared decision-making processes.

J.L. Castaneda: None.

P19.12C
Genetic Counsellors working with young people: overview of current needs and glimpse forward

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Introduction: Genetic counselling has been consistently shown to increase understanding of genetic disorders and the implications of genetic testing, manage emotional distress and stigma, promote empowerment and patient autonomy in choosing screening or treatment options. The overwhelming evidence is primarily based on adult data. Genetic counselling of young people is less reported on and brings with it additional challenges. To this date we know little about genetic counsellors' needs, knowledge and confidence when working with children and adolescents.

Method: An online survey assessing genetic counsellors' knowledge and skills used in the counselling process, self-efficacy in working with children and adolescents and access to training specifically for working with age group, was sent to European and national professional organizations representing 20 countries.

Results: At the time of the abstract submission data is still being gathered. A preliminary analysis shows that genetic counsellors' self-efficacy in conducting genetic counselling sessions with young people is rather modest; there is overwhelming consensus on the need for more theoretical and practical training. Specific knowledge about child development and communication strategies were among the most frequent needs mentioned by genetic counsellors.

Conclusions: Having a clearer insight into genetic counsellors' needs when working with this age group can be particularly informative for training programmes.

Focusing on developing specific skills and knowledge, drawing on the knowledge of pediatrics, developmental psychology and counseling, and empowering genetic counsellors to acknowledge their skills when working with children and adolescents are key aspects in training and supervision.

M.M. Radu: None. **A.S. Baban:** None. **R. Moldovan:** None.

P19.13D

Insight into the impact of genetic counselling on English and Urdu speaking Pakistani families affected with an autosomal recessive disorder living in the UK North West

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Introduction: Cousin marriages are common in the UK South Asian population and consanguineous couples are at increased risk of having a child with an autosomal recessive disorder. This study explored views around consanguinity and the utilisation of genetic information following genetic counselling of both English and Urdu speaking South Asian women who had children with an autosomal recessive condition.

Methods: Participants were ascertained through purposive sampling. Face-to-face semi-structured interviews were conducted with English and Urdu speaking genetic counsellors. Transcripts were analysed using thematic analysis.

Results: Twelve participants took part in interviews; 6 English speaking and 6 Urdu speaking. Most (92%) were in consanguineous marriages. Similar themes included; effect of genetic counselling on reproductive decision making and the perception of a cultural shift in the practice of cousin marriage. Differences in themes included; genetics being an abstract concept among Urdu speaking participants and a greater willingness to share genetic information among English speaking participants.

Conclusion: The findings of this study provide a valuable insight into an insufficiently researched minority group and has implications for how services are delivered and developed for these families. This study confirms that families do engage and utilise genetic services. However, it highlights the essential need for greater awareness towards the cultural sensitivities and attitudes towards genetics for service development among this ethnic population.

V.A. Leach: None. **N. Khan:** None.

P19.14A

Utilisation of genetic counselling services in Germany before and after the enactment of the German Gendiagnostikgesetz (GenDG) – Results of the GenBIn study

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Introduction: The GenDG stipulates the requirements of good practice in regard to safety, patient rights to know, patient rights to decide and requirements for genetic counselling. A guideline on the requirements for contents and qualification in genetic counselling within the scope of each medical specialty came into force in 2011.

Methods: Development of a database (GenBIn) that provides both, baseline data on utilisation of genetic counselling services prior to the GenDG (status quo ante) and data on utilisation after the enactment.

Results: 26 genetic counselling centres volunteered to collect data on in person counselling cases in 2011, 34 centres provided data for counselling cases seen in 2016/2017. In total the GenBIn database contains data on 5256 cases. Compared to 2011 the relative share of referrals by obstetricians&gynecologists has decreased (83%/63%), whilst referrals by other specialists (15%/27%) and self-referrals (2%/10%) have increased in private practice. Referrals for prenatal counselling have decreased, counselling for familial cancer and other familial disorders has increased. Workload and mean waiting time per case have increased.

Conclusions: The data document a rising demand for genetic counselling services and a shift in indications for referral. Arguably, the observed changes are due to a multiplicity of causes and are not attributable solely to the GenDG. Advances in genetic testing technology, growing awareness among healthcare providers and among patients (increased self-referrals) are likely to contribute together to higher patient volumes, to increased workload and to capacity constraints for genetic counselling services. Funded by German Federal Ministry of Health ZMVi1-2515-FSB-7

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P19.15B
Development of a genetics education program: equipping medical professionals for the genomics era

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Advances in molecular diagnostic technologies and greater integration of genetic testing into the diagnostic process have required the modern healthcare provider to become literate in genomics. Many physicians report lack of knowledge and confidence as barriers to offering appropriate genetic counselling. In response to this, ahalf-day interactive workshop was developed to provide existing healthcare professionals with current knowledge in field of genetics.

The educational program was tailored to equip providers with an awareness of the benefits, limitations and risks of available genetic testing. Course components included basic genetic concepts, current testing methodologies, interpretation of results and local legal frameworks regulating genetic testing. Through case studies, providers also gained an understanding of medical, social, ethical and legal issues surrounding genetic testing.

Since 2018, the workshop has been run twice, with 2 more runs planned in 2019. Participants include more than 90 healthcare providers from various healthcare professions. A pre- and post-course evaluation was conducted to assess knowledge improvement and self-reported confidence in ordering and interpreting genetic test results. There was an improvement of a pre-course score of 6 to post-course score of 9 out of 12 questions. However, participants generally did not report changes in their level of confidence in ordering or interpreting genetic test results. The genetics education program is effective in improving the level of genetic knowledge amongst healthcare providers. It will be useful in future to explore the factors that increase provider confidence in ordering and interpreting test results so as to empower healthcare providers in this genomics era.

B. Cham: None. **T. Ting:** None. **Y.M. Bylstra:** None. **A. Lai:** None. **S.M.D. Dominguez:** None. **S. Jamuar:** None.

P19.16C
Is follow-up our future? A focus on changing roles in genetic counselling

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Genomic technology and the expansion of mainstreaming mean that increasingly complex test results are being delivered in a wide range of medical settings. Genetic counsellor (GC) involvement will be critical to successfully navigate this situation, ensuring that patients understand and benefit fully from their tests. GC expertise will be important in supporting families, especially in the face of uncertain or evolving results.

Cross-sectional survey data (Family Web study n=286) supports the importance of a trusted expert who can balance these different roles. Data indicate that, in a population of patients at high risk of bowel cancer, the diagnosis had a profound effect on patients and their families: participants wanted more support, ideally from a care co-ordinator; plus information on a range of topics and a positive message to give their relatives. Key issues identified included: gene-specific risks, accessing adequate cancer surveillance, and advice on how to reduce cancer risk through lifestyle.

Pressures to meet clinical targets, combined with increasingly robust GC training, have resulted in a greater prevalence of GC-led clinics for diagnostic and predictive testing. It seems likely that this will be matched by an increased emphasis on follow-up. GCs possess the necessary range of skills to provide ongoing liaison services, assisting with the two-way communication that is necessary for managing uncertain results over time. Successful genomic services depend on patients successfully assimilating information; GCs may be uniquely placed to support this across a range of settings.

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P19.17D
Teaching Clinicians Practical Genomic Medicine – Our 7 Year Evaluated Experience

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Introduction: With introduction of various genomic technologies into the clinic, physicians must be proficient in genomics. Training of physicians in genetics is offered in programs across Europe and USA, but there is need for standardized, effective program-models. We sought to evaluate effectiveness of our genomic-education-model.

Methods: Our program served physicians from various levels of training and disciplines. We used 11 different program-formats (8-44hrs, 10-35participants), most frequently our "extended-format"- week-long course combining lectures, educational-movies, interactive tests, and practical exercises. Lecturers were dedicated team of 15 geneticists. Pretest exams assessed baseline knowledge; posttest exams after program evaluated improvement. Pre- and posttest scores between different groups were compared using t-tests (SAS-institute version9.4).

Results: Over 7 years, 774 clinicians attended 35 programs (924 hours total) over 7 years, 334 participants completed "extended-format". Knowledge improved greatly for all participants (mean improvement 15.05 points, $P<0.001$). Residents started with better knowledge than specialists (pretest score 66.3±17.3 vs. 58.7±16.6, respectively, $p=0.002$). Both groups improved knowledge significantly, by similar magnitude, with specialists "catching-up" (post-test 79.1±17.2 vs. 75.7±15.9, NS). The trend was same when comparing fellows and subspecialists (pre-test 70±18 vs. 59.4±16.4, $p=0.007$, post-test 78.6±16.4 vs. 73.2 ±17.7, NS). Specialists who completed training within last 10years had significantly higher scores than those practicing >10years (before and after program). Despite differences in scores, degree of improvement was equivalent for physicians from various disciplines.

Discussion: Our program is effective in improving genomic knowledge of clinicians, regardless of practice years and disciplines. It could serve as a model for improving genetic skills of medical-professionals.

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P19.18A
Greatly expanded new 2019 versions of free educational Clinical Genomics apps for smartphones & tablets: international uptake and further development

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Introduction: Android & iPhone (iOS) educational smartphone apps that explain (and test knowledge of) commonly used genomics and bioinformatics terms and acronyms, were developed by the authors & recently greatly expanded.

Methods: Further development has included iOS12-compatibility and doubling the number of terms and concepts explained. The new total of 42 includes: databases (e.g. gnomAD, ExAC & COSMIC), genome projects, constraint metrics (e.g. pLI & Z scores), polygenic risk scores, third-generation sequencing & biological concepts e.g. topologically associating domains & NMD. These Clinical Genomics apps are being made available via the Apple and Android app stores (search: "Clinical Genomics"). They provide immediate clear explanations (often difficult to find elsewhere) of genomics-related terminology (such as "MNPs", "BED", "PED", "FASTQ", "BAM", "CRAM", "GVCF", "BED files", "GATK" and "BWA").

Results: Highly positive feedback has been received from physicians, genetic counsellors, laboratory scientists and students. The apps are used by professionals, who increasingly receive complex genetic laboratory reports requiring interpretation 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 60 countries and were shortlisted for a national higher education innovation award.

Conclusions: Although creating these multi-platform apps was complex and time-consuming, they have thankfully been found very useful by many individuals. The authors would welcome feedback and suggestions for additional terms/concepts to include. Author APT, a medical student, is grateful to have been a recipient of a Young Enterprise Scotland Innovation Award 2018.

A.P. Tobias: None. **E.S. Tobias:** None.

P19.19B
The Influence of Natural Sciences on the Development of Human Genetics as a Medical Field in the Federal Republic of Germany

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Introduction: In 1953, biologist JD Watson and physicist FHC Crick published the molecular structure of DNA. Together with physicist MHF Wilkins they were awarded the Nobel Prize in Medicine or Physiology in 1962. Natural scientists added more fundamental principles to human genetics in the following decades.

Materials and Methods: Basis are the results of the DFG-Project of H. Petermann. Based on those data, we are conducting interviews with human geneticists, natural scientists and physicians. The focus is on the role of scientists of various fields (medicine, natural sciences, anthropology) in establishing human genetics at universities in the FRG.

Results: 1. In the 1950s physicians and philosophical anthropologists dominated the institutes that did mainly paternity tests. Those were paid by the courts that instructed them. 2. In 1960 the Scientific Council of FRG (*Wissenschaftsrat*) forced the establishment of Human Genetics Institutes as a field of medicine. 3. Since the beginning of the 1970s National Health Insurances paid for genetic counseling by physicians, according to the *German Scale of Fees for Doctors* (GOÄ). 4. Therefore, two departments were established at Human Genetics Institutes: for diagnostics by natural scientists and for genetic counseling by physicians. 5. Lectures were given by physicians and natural scientists.

Conclusions: The role of physicians and natural scientists at Human Genetics Institutes is determined by two facts: historical reasons and reimbursement of expenses. Therefore, head of Human Genetics Institute at medical faculties is always a specialized physician. For changes, there must be a revolution of the system.

H.I. Petermann: None. **T. Liehr:** None.

P19.20C
The first *CDH1* founder mutation in Portugal: Risk assessment and clinical management

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Introduction: Germline *CDH1* mutations cause hereditary diffuse gastric cancer (HDGC), characterized by early-onset multigenerational diffuse-gastric-cancer (DGC) and lobular-breast-cancer (LBC). Risk-reduction-gastrectomy (RRG) and –mastectomy (RRM) may be life-saving if performed in asymptomatic carriers. In Northern Portugal, we identified 7 HDGC-unrelated families sharing the c.1901C>T variant, raising the hypothesis of a founder-effect. We calculated mutational-age, characterized tumour spectrum and analysed management output in the extended pedigree (n=114 individuals).

Materials and Methods: RNA-based analysis was performed to prove variant deleteriousness. *CDH1* c.1901C>T variant carriers and non-carriers were used to estimate the founder-effect age by decay of haplotype sharing methods. Carriers' clinical data were collected as they were followed in high-risk consultations.

Results: Germline RNA from c.1901C>T carriers demonstrated production of a single truncated transcript. All carriers shared the same ancestral haplotype in a 6.8 Mb/5.1 cM region. The time to the most recent common ancestor was estimated to 489.64 [CI: 445-10,900] years. Following death of 5 DGC probands (3F:2M), aged 18-35, we identified 48(24F:24M) additional carriers. In female-carriers, 9 underwent RRG, 7 both RRG and RRM, and 8 endoscopic/MRI surveillance. In male-carriers, 10 underwent RRG and 14 endoscopic surveillance. The application of a strict surveillance/prophylaxis protocol, following positive genetic-testing, allowed DGC (n=4) and LBC (n=2) early-detection and sub-clinical disease detection in RRG and/or RRM surgical specimens (DGC=19, LBC=4).

Conclusion: We identified the first Portuguese *CDH1*-related founder-effect with ~500 years. Reaching out to 48 carriers from 7 families allowed to adopt appropriate risk-reducing surgeries and/or surveillance, so far without further cancer-related deaths. *Equal contribution

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P19.21D
Attitudes of Genetic Counsellors towards counselling young adults through predictive testing for Huntington's Disease (HD)

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Majority of individuals who seek a predictive test for Huntington's disease (HD), have been primarily found to be young adults (YA) (18-30 years old). With no current direct medical benefit to testing, as well as the uncertainty in the severity/age at which symptoms may appear, the emotional, ethical and other psychological consequences associated with the test have been found to be increased. The

importance of genetic counselling and support for at-risk individuals remains crucial to enable a better understanding on the impact predictive testing can have on one's life. However, YA have previously reported lack of emotional support and a communication barrier during these consultations. As there is limited research conducted on the explicit experiences of genetic counsellors with predictive testing for HD, it is difficult to establish why this gap in support and communication exists. The aim of this study was to gain a better understanding of experiences of GCs whilst counselling at-risk young adults for predictive testing for HD, as well as explore the HD service provision in Scotland. Nine genetic counsellors from three regional centres in Scotland (Glasgow, Aberdeen and Dundee) were interviewed using a semi-structured interview guide. Thematic analysis identified three key themes: challenges faced with young adults and Huntington's disease, aspects of the pre-symptomatic predictive testing protocol and the relationships with other health care professionals. Being made aware about these experiences can highlight gaps in training and support for genetic counsellors as well as other healthcare professionals.

U. Savania: None. **S. Lesley:** None.

P19.22A
How to inform relatives at risk? Attitudes of 1.379 patients, relatives and members of the general population

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Introduction: The uptake of predictive DNA testing in families with a hereditary disease is less than 50%. Current practice often relies on the proband to inform relatives about the possibility of predictive DNA testing, but not all relatives are informed adequately. To enable informed decision-making concerning predictive DNA testing, the approach used to inform relatives needs to be optimized. This study investigated preferences of patients, relatives, and the general population on how to inform relatives at risk of autosomal dominant diseases.

Methods: Online surveys were sent to people with an autosomal dominant neuro, onco or cardiogenetic disease and to their family members via patient organisations (n=379) and to members of the general population via a commercial panel (n=1000).

Results: Attitudes of the patient and population samples generally corresponded. A majority of participants believed that initially only first-degree relatives should be informed, following the principles of cascade screening. Most participants also thought that patients and healthcare professionals (HCPs) should be involved in informing relatives, and a large proportion believed that HCPs should contact relatives directly when patients are unwilling to inform, for untreatable and treatable conditions. Participants from the patient sample were of the opinion that HCPs should actively offer support.

Discussion: Our findings show that both patients and HCPs should be involved in informing relatives at risk of autosomal dominant diseases and suggest that relatives' 'right to know' was considered a dominant issue by the majority of participants. Further research is needed on how to more actively inform at-risk relatives.

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P19.23B
Experiences with transition of gene panel DNA-diagnostics from clinical geneticists to treating physicians in breast cancer patients

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Introduction: In many European countries requesting DNA-diagnostics for hereditary breast cancer has traditionally been the field of clinical geneticists. Since test results can now be available within 2-3 weeks, these are increasingly considered for determining treatment options in breast cancer patients. Treating physicians therefore increasingly refer eligible, recently diagnosed breast cancer patients for rapid DNA-testing to the clinical geneticist. To facilitate timely test results, we aimed to shift counseling and requests for rapid DNA-diagnostics for these patients from clinical geneticists to treating physicians and evaluate this transition towards so-called 'mainstreaming'.

Methods: The project was initiated by the departments of Clinical Genetics from Maastricht University Medical Center and Radboud University Medical Center, Nijmegen, The Netherlands. Regional hospitals were included one-by-

one from July 2018 onwards. Treating physicians were asked for their needs and barriers to participate. DNA-diagnostics consisted of gene panel analysis for *BRCA1, BRCA2, PALB2, CHEK2* and *ATM*.

Results: In the start-up phase, treating physicians requested hands-on information, for which the website www.DNAfirst.nl was developed. Barriers included time investment during consultation and perceived incompetence of providing breast screening advice for family members. Therefore, clinical geneticists joined multidisciplinary meetings. Up to February 2019, nine hospitals were included, 65 requests for DNA-diagnostics made and three pathogenic mutations found (4.6%) for which patients were referred to a clinical geneticist. Structured evaluation of quality of care and experiences of doctors and patients will follow shortly.

Conclusion: Transition of rapid gene panel diagnostics from clinical geneticists to physicians treating breast cancer patients appears to be feasible.

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P19.24C
Parental perceptions to recurrent chromosomal microarray findings and their implications

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Introduction: Following the American College of Medical Genetics (ACMG) guidelines in 2010, chromosome microarray analysis (CMA) has become the baseline genetic test performed in individuals with neurological and cognitive impairment which may often occur in association with multiple congenital anomalies. However, there is scarce information regarding the families' comprehension of microarray results indicative of recurrent chromosomal micro-deletion/ duplication findings. This is especially true in cases where microarray testing identifies parents to be carriers of similar chromosomal alterations as their child and where parents may/may not have some clinical manifestations of their own.

Method: This is a qualitative exploratory study, where semi structured interviews were conducted with four mothers of children identified to have recurrent chromosomal micro-deletion/duplication syndromes. The interviews were transcribed verbatim and analysed using Interpretative Phenomenological Analysis (IPA).

Results: Three superordinate themes emerged from the study: validation of a diagnosis, response to unexpected test results & acceptance patterns and thirdly, impact of results.

Conclusion: This study identified that all the parents interviewed valued the genetic diagnosis provided by microarray testing. Acceptance patterns of results differed based on personal family differences. However there still existed some uncertainty regarding their child's future. Interventions to help parents adapt must also include imparting information about what is known and what is yet to be known about these rare and variable conditions. Parents may benefit from follow up care in order to keep abreast of latest developments.

S. Devaiah: None. **S. Douzgou:** None. **M. Bottomley:** None.

P19.25D
Mucopolysaccharidosis type IVA (Morquio syndrome) A rare case report

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Motivation: One of the rare inherited diseases is the mucopolysaccharidoses disorders (MPS) associated with accumulation of glycosaminoglycan in several organs, leading to abnormalities in musculoskeletal, respiratory, cardiac, neurological, ophthalmological, otolaryngological, and gastrointestinal. Nowadays, an increasing number of patients with MPS are reaching adulthood and are involved in family planning due to improvements in diagnosis, multidisciplinary care, and therapies such as enzyme replacement therapy and hematopoietic stem cell transplantation. Knowledge on skeletal deformity in MPS remains relatively uncommon and includes a few case reports. To assess more, we present a case report on musculoskeletal deformity in one subject with MPS.

Case: This case revealed that subject with MPS has generalized osteoporosis, Degenerative Joint Disease (DJD) of both hips, general skeletal deformity alongside normal mental status. He is 26 years old and the parents were relatives (1st cousin). He had one healthy younger sibling. On physical examination, disproportionate short stature, skeletal dysplasia, osteoporosis, prognathism, negative ulnar variance, angle blunting and ulnar styloid were detected. Intelligence is normal and there is no direct central nervous system (CNS) involvement. In the present research work, C.181C>T (p.Arg61Trp) variant of GALNS gene was

found through whole exome sequencing and the mutation was confirmed using Sanger sequencing method, which is associated with MPS4A phenotype.

Conclusions: Pathogenic variant in the GALNS gene associated with hereditary Mucopolysaccharidosis IVA, (MPS4A, Morquio syndrome A) which encodes N-acetylgalactosamin-sulfate sulfatase, may be useful as a biomarker to early diagnosis and treatment.

M. Mahdavi Amiri: None. **A. Khoshaeen:** None.

P19.26A

Multiple Endocrine Neoplasia type 1 (MEN1) associated moderate breast cancer risk: Women's attitudes towards the introduction of additional breast screening

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Background Multiple Endocrine Neoplasia type 1 (MEN1) is a rare inherited condition characterised by a lifetime risk of developing numerous tumours at different sites of the body. The main clinical manifestations include tumours of the parathyroid glands, pancreas and anterior pituitary gland. Recent research suggested that female patients with MEN1 might also be at an increased risk of early onset breast cancer. Therefore, there is a potential need to introduce early breast screening into the surveillance programme of this group of patients. The purpose of this study was to explore attitudes towards the introduction of additional breast screening among women with MEN1 who were mainly unaware of this risk and already undergoing intensive screening investigations.

Methods: Seven women with molecular diagnoses of MEN1 were recruited through Clinical Genetics at the Queen Elizabeth University Hospital (Glasgow). Semi-structured qualitative interviews were conducted. Interviews were transcribed verbatim and analysed using thematic analysis.

Results: Three main themes underpinning the attitudes about additional breast screening emerged: positivity, concern and information needs. All participants were positive about the introduction of additional breast screening. However, a fear of cancer, risk misperceptions and lack of information were identified as major concerns affecting their willingness to be screened.

Conclusions: The positive attitude of women with MEN1 supports the feasibility of introducing additional breast screening into the surveillance program of this group of patients. Emphasis should be placed on the risk communication process in order to facilitate informed decisions alongside maintaining the wellbeing of these patients.

A. Esteve Garcia: None.

P19.27B

Next Generation Sequencing in health care and clinical research: attuning all steps

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Responsible implementation of NGS in health care involves different disciplines which need to communicate and cooperate. For instance, while clinicians ask informed consent for NGS, lab-technicians performing DNA sequencing and analysis do not always know what exactly was consented to. Contrariwise, those who are developing consent forms and/or performing consent conversations with the patient, require information from all other parties involved in later steps of the NGS process.

To elucidate the key steps in the communication between the different parties involved in NGS in health care and clinical research, an expert meeting was organized by the National Consortium of Ethical, Legal and Social Implications of Personalised Medicine (ELSI-PM) in the Netherlands.

Ideally, it should be clear to all parties what exactly is consented to. Transparency is required about e.g. how long the samples and the data can/should be stored, what (unsolicited) results should be communicated to the physician and/or patient, possibility of data-sharing between physicians and/or researchers, the types of research that can be conducted, and conditions for data de-identification.

Furthermore, the content of the informed consent forms and conversations should be aligned with the requirements for effective and responsible execution of each of the next steps in the NGS process.

This requires not only clear definitions of roles and responsibilities of the different parties involved, but also

agreement on, and facilitation of, the sharing of information across disciplines, including the content of the consent.

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P19.28C
Developing clinical bioinformatics online learning using agile and reusable principles

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Introduction: We present an online learning course in clinical bioinformatics developed using an agile approach and reusability of components. The University of Manchester delivers face-to-face post-graduate taught courses to trainee Clinical Bioinformaticians in the UK National Health Service. A recent MOOC (Massive Open Online Course) introducing this subject area, and market research has demonstrated the wider demand for skills and knowledge development in this field.

Materials and Methods: We used agile processes for course development including user storyboarding, prioritised development sprints and iterative development. We took a reusable approach to development of teaching materials to make them useable in other contexts.

Results: Using Laurillard's conversational framework the course was divided into sections and discussion activities designed. Each section was further divided into learning objects which were developed iteratively by subject matter experts using a range of teaching approaches. Team meeting were used to prioritise each development sprint. Learning objects were designed to be non-context specific and thus reusable: weekly topics were formed by contextualising a series of learning objects using text, videos and interactive activities using the Articulate Rise 360 platform. User Acceptance Testing provided feedback on the completed course.

Conclusions: This approach has made course development more flexible, easier to manage and innovative. Initial overheads for development of online material are high, but enable easier maintenance of the course. Development of a library of reusable learning objects (RLOs) will allow faster and easier development of further courses. Details of the course can be found here: http://bit.ly/clinicalbio

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P19.29D
Pre-genetic counseling telephone interviews (PTI) and routing: an efficient procedure for an accessible, appropriate, accelerated and affordable genomic medicine

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We previously described a new procedure, based on pre-genetic counseling telephone interviews (PTI) followed by routing of patients, in order to address the challenges of the exponential demand for genetic counseling, especially for patients suspected to present with hereditary breast or ovary cancers (HBOC). We provide here an update of the procedure extended to a total of 4272 patients suspected of HBOC, hereditary colorectal or renal cancers. After the PTI scheduled within 14 days and performed by genetic counselors, a pre-genetic counseling file (including tumour data and status of treatment and number of affected and unaffected first- and second-degree relatives), is established. The PTI is then submitted within 7 days to the routing performed by a cancer geneticist with 3 possible **conclusions:** (i) priority face-to-face genetic counseling (FTFGC), with a cancer geneticist, if the indication is confirmed and genetic analyses have potential immediate therapeutic impacts, (ii) non priority FTFGC with genetic counselors if the indication is confirmed, but genetic analyses have no immediate therapeutic impacts and (iii) non indication for FTFGC. For 1525 patients (35.7%), FTFGC was considered not justified and approximately 10% of the patients benefited from priority FTFGC. The human resources required for the genetic counseling, using this procedure, was estimated per 1000 index cases at 0.12 FTE secretaries, 0.62 FTE genetic counselors and 0.08 FTE medical geneticists, confirming our previous estimate. This simple strategy, based on PTI and routing, allows an accessible, appropriate, accelerated and affordable genomic medicine, especially in the field of cancer genetics.

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P19.30A

Capacity building and empowerment program in European Joint Program on Rare Diseases: time to address unmet needs in rare disease research education and training

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Rare diseases (RD) are conditions with prevalence of <5/10,000 inhabitants, ~80% of RD have genetic etiology. Despite significant developments in recent years, major challenges remain including delayed RD diagnosis, insufficient treatments and difficult access to appropriate care. In view of current massive generation of new data, introduction of omics into care practices and ongoing RD care structuration in European Reference Networks (ERN), there is crucial need to develop RD research capabilities, to spread know-how and standards in RD research among stakeholders. Recently launched European Joint Program on Rare Diseases (EJPRD) encompasses comprehensive and cohesive capacity building and empowerment program addressing developing needs of RD research community in terms of content, geographical coverage, targeted audiences. Existing resources will be pooled and novel will be created to tackle areas as data management, orphan drug development, HTA/regulatory processes, scientific innovation/translational research, best practice guidelines and ERN cross-cutting themes/needs. The face-to-face capacity building activities will train ~1,500 participants over 5 years, while first-in-class online training program has the potential to reach unlimited number of trainees in the long term. The program will target various groups of stakeholders - researchers, practitioners, patients/their representatives. Specific attention will be given to patient-centredeness in research and the needs of ERN and EU13 countries. Increased accessibility of trainings will be secured through course rotation, fellowships and open access to some online resources. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825575, EJPRD, 2019-2023.

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P19.31B

Reproductive genetic counseling and management of hemoglobinopathies carriers couples

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Introduction: The premarital hemoglobinopathies screening associated with genetic counseling and genetic testing offer people at risk the opportunity of making informed decisions on marriage and reproduction.

Material and Methods: Preconceptional counseling was offered for couples at risk for haemoglobinopathy offspring, referred to Genetic Clinic at University Medical Center Bahrain, from 2016 till 2019. 21 couples were counseled, genetic testing confirmed the carrier status and those who opt out for preimplantation genetic diagnosis (PGT-M) were referred to In Vitro Fertilization Clinic (IVF). PGT-M of the particular mutations identified in the couples were performed and pregnancies were initiated with the healthy and non-carrier selected embryos.

Results: 38% couples were carriers of sickle cell, 33.33% carriers of sickle cell and beta thalassemia, 28.57% carriers of beta thalassemia, 4.76% carrier of sickle cell and hemoglobin D and 4.76% carrier of beta and alpha thalassemia. 66.66% of the couples were non consanguineous, 76.19% were married and 40% of them had already one affected child. 57.14% (12/21) couples underwent the IVF +PGT-M, 58.33% (7/12) for Sickle Cell Disease and 41.66% (5/12) for Sickle Cell/beta thalassemia disease. Pregnancy was achieved in 50% (6/12) couples, 2/6 miscarried, 3/6 have an ongoing pregnancy in 1/6 gave birth to a healthy child.

Conclusions: There is a high hemoglobinopathies carrier status in non-consanguineous couples. Genetic counseling allows the carrier couples to understand their risk and helps them in taking their best reproductive decision. PGT-M was the main option and had a good pregnancy rate.

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P19.32C

BrecanRisk: Stratification of high-risk women to suffer from breast cancer

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Introduction: During last years, the main genetic (SNPs) and phenotypic risk factors in breast cancer have been described. In this context, we have developed Brecan Risk algorithm, in order to integrate these factors and assess the risk throughout women's life.

Material and Methods: In the first phase, the genotypic stratification was validated in a cohort of 930 healthy women and 1202 patients, analyzing 120 SNPs related to breast cancer susceptibility from peripheral blood DNA. In the second phase, the algorithm was validated in a cohort of 790 women by integrating genotypic and phenotypic data (family history, previous biopsy, breast density, age of menopause, age at first pregnancy). In two independent retrospective cohorts followed up for 2-15 years, BrecanRisk was calculated and correlated with breast cancer development.

Results: The genotypic analysis separated case-control populations and stratified them according to the risk based on the genotype-phenotype combination. 67% of the women were classified as low-medium risk (0-1.67) while the remaining 33% were classified as moderate (1.67-2.49) or high risk (>2.49). In the first retrospective cohort (170), 9 women developed cancer, being 80% of them stratified as moderate-high risk. This result was supported in the second cohort recruited through the screening program (157).

Conclusions: The BrecanRisk algorithm efficiently stratifies women according to their risk to suffer from breast cancer depending on the individual genotype and phenotype. Based on our retrospective studies, the correlation between a high-risk result and subsequent cancer is 80%.

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P19.33D

Working in Cambridge, UK and Brisbane, Australia: A comparison between 2 Clinical Genetics services in the United Kingdom and Australia

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Clinical Genetics services have evolved rapidly in a relatively short period of time. They have had to adapt to technological change, increasing demand, increasing awareness by other clinicians and the public of the genetic burden of disease. There have been different models adopted depending on the healthcare system, funding models, resources available, training, personnel and geography.

These services are increasingly requiring new and innovative ways of working to meet demand and overcome the significant shortage of trained health professionals in Clinical Genetics. Comparing and contrasting delivery of these services between countries is a way of benefitting from innovations which have worked elsewhere.

I am a trained Clinical Geneticist from Cambridge, UK, and worked in a similar role in Brisbane, Queensland, Australia for a year. I will describe the pathway to be recognised as a Clinical Geneticist in Australia and the similarities and differences in the services.

There were significant similarities with the clinical work in both centres however triage, organisation of the patient pathway, use of IT software and role of Genetic counsellors was different. There were variable numbers of staff per million population.

As an example, due to the large geographical area covered by the statewide service in Queensland, telehealth utilisation enabled access in remote areas and also to enable subspecialist care to be delivered. Due to the increasing demand and lack of resources, the Cambridge department had transferred some roles to administrative staff to allow

the Genetic counsellors and Doctors time to focus on their specialist skillset.

S.G. Mehta: None.

P19.34A

Perspectives in genetic counseling for spinal muscular atrophy in the new therapeutic era

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Introduction: Genetic diagnosis and counseling in Spinal muscular atrophy (SMA) present several challenges including the existence of carriers that are undistinguishable of non-carriers and the report of patients with very mild manifestations and even asymptomatic that are discovered when a full symptomatic case appears in the family. Younger asymptomatic siblings of symptomatic SMA patients are usually never tested until adolescence or adult life. However, following regulatory approval of the first tailored treatment for SMA, the prospects for care of these patients have changed as early testing would change proactive measures and opportunities for these patients.

Methods: Different aspects of genetic counseling that could potentially be influenced by new advanced therapies are explored. Among them are considered: 1) Risk of recurrence and diagnosis of carriers in the family 2) Reproductive options 3) Phenotypically concordant and discordant brothers 4) Prevention levels 5) Predictive genetic markers of disease evolution 6) Early intervention: criteria for neonatal screening and presymptomatic treatment. Two clinical cases are deeply analyzed.

Results: In all the aspects analyzed there were potential substantial changes regarding attitudes and decision-making. Of particular relevance is the fact that at present the SMA would fulfill the total of the 10 criteria of Wilson and Jungner to be considered a candidate disease for neonatal screening.

Conclusions: The approval of effective advanced therapies is changing the way in which the communication and genetic counseling is assumed in SMA. It is essential that professionals be aware of these advances as they change the perspective of this disease.

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P19.35B

The discussion of uncertainty concerning multigene panel testing during cancer genetic counseling. An observational study

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Introduction: Pre-test counseling about multigene panel testing involves an increased level of uncertainty. Ideally, counselees are fully informed about uncertainties to make an informed decision about whether or not to perform such a test. It is presently unknown whether and how uncertainty is discussed during initial cancer genetic counseling. We therefore investigated in which counselors discuss uncertainty concerning multigene panel tests, address counselees' uncertainties, and whether their manner is associated with counselors' characteristics.

Materials and Methods: Videotapes were made of counselors discussing a multigene panel test with a simulated patient (SP). SPs represented a counselee who had had multiple cancer types. Before and after their consultation, counselors completed a survey. Counselors' uncertainty expressions, initiative- and framing of expressions, and counselors' verbal responses to SP's scripted uncertainty expressions were coded.

Results: Counselors (N=29) expressed uncertainties mainly regarding scientific topics (94%) and on their own initiative (95%). Most expressions were framed directly (77%; e.g. *We don't know*), and non-valenced (59%; i.e. without a positive or negative value). After SPs expressed uncertainties, counselors mainly responded by explicitly referring to the uncertainty (69%) without providing space for further disclosure (66%). More experience with genetic counseling led to a decrease in space for further disclosure of SPs' uncertainties ($p<.02$).

Conclusions: Mainly communicating scientific uncertainties, and using mostly space-reducing responses, raises the question whether enough attention is paid to counselees' personal uncertainties allowing them to disclose their concerns during initial genetic counseling.

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P19.36C
Whole exome sequencing in Romania - beginnings and challenges

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Introduction: Genomic technologies are an integral part of the genetic investigation toolkit in many areas of medicine. While genomic data accumulates at a very fast pace, its availability and the specifics of the local genetic landscape are among main challenges encountered by the biomedical or clinical geneticist. We present our preliminary data on the implementation of whole exome sequencing (WES) technology as a clinical investigation tool in our Romanian patients.

Materials and Methods: Twenty two patients with neurological, metabolic, cardiac, and skin disorders were referred to our laboratory for WES or panel sequencing between June 2017 - January 2019. Ion GeneStudio S5 System (Thermo Fisher Scientific) and Ion Reporter platform were used.

Results: Eleven pathogenic or likely pathogenic mutations associated with part of/complete clinical phenotypes were found in *ACADS, ACADVL, ALOX12B, AP4B1, APOE, GJB6, IQSEC2, PHKB, TBCK* genes. 3 VOUS in *ACADVL, GK, KCNMA* genes possibly causative for the clinical presentations of patients were also described. Thus, mutations explaining the patients' phenotypes were found in 7 cases, indicating an overall detection rate of 30.43%; in 2 more cases only part of the phenotype was clarified.

Conclusions: Genetic diagnosis is becoming increasingly accessible throughout the Western world. However, small, local clinics beyond this geographical space encounter specific challenges when embarking in WES, such as the genetic particularities of the local populations, the need to establish an open dialogue within multidisciplinary medical teams, competition with larger Western companies and, quite often, the lack of patient financial assistance from local health insurance systems.

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P19.37D
Memory game in the learning about X-linked diseases

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Introduction: The inheritance patterns of X-linked disorders had been easily identified due to unequally distribution of the chromosome X between women and men. Like the other monogenic diseases, X-linked disorders are categorized into dominant and recessive. In order to teach about this inheritance pattern of diseases, it has been created a memory game, which the main purpose was to inform, in a playful way, their characteristics.

Materials and Methods: This memory game contains 24 cards, split in 12 pairs. Each pair has a card with informative text and another with an illustrative figure about the corresponding text. The game needs two players that will use memory and logic capacities. As the game progresses, the player must use memory to store information contained in the card and his position in the game, winning the player that achieves a larger number of correct pairs.

Results: We used eight players, divided into four pairs. They had a few knowledge about the subject and, during the game, were correlating the texts with the figures. In the end, the players were able to correctly relate the information contained in the cards with the respective figures.

Conclusion: The memory game proved to be efficient in acquiring knowledge about the theme, brought in a playful and easy-to-learn way. By relating the information contained in the texts with the images, the players presented a greater understanding about the features of X-linked diseases.

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P20
Psychological and social issues in genetics

P20.01A
Cognitive and psychosocial profile in Silver-Russell syndrome: a first study in adults

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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 (Wakeling et al., 2017). In 50-60% of cases, SRS is caused by an epimutation of the region 11p15. Currently, no studies have been published on the assessment of cognitive, psychological and behavioral particularities of adults with SRS.

Methods: An evaluation of cognitive and psychosocial profile was performed in 11 patients (7 men), aged 18 to 39 years (M=24.36 years). All patients had an epimutation of the region 11p15 IGF2/H19 region. Ten patients had a bachelor's degree and 7 completed a higher education. 70% were treated with growth hormone and 90% have benefited from speech therapy and/or psychological care.

Results: Mean overall IQ score was 95.82 (17.65 SD). Verbal comprehension index was on average higher than other indexes (mean 109.55; 18.17 SD). We have found a trend link between birth weight and Processing Speed Index and between birth head circumference and Perceptual Reasoning Index. The abilities to concentrate were considered very low (score below 84) in 4 patients. Self-esteem was also very low (score below 33) in 4 patients. Internalizing problems score was higher in SRS group than in the normative control group (p=0.032).

Conclusion: Overall adults with SRS had normal intellectual efficiency. In our group, some specific cognitive and psychosocial profiles were observed. These results improve knowledge about SRS and point to the importance of early intervention and multidisciplinary care from childhood to adulthood.

M. Burgevin: None. **A. Lacroix:** None. **A. Toutain:** None. **D. Martin-Coignard:** None. **M. Vincent:** None. **C. Crosnier-Schoedel:** None. **V. Coutinho:** None. **I. Netchine:** None. **S. Odent:** None.

P20.02B

Genetic risk information (*APOE*) as a lifestyle change motivator in Finland - Description of the participant population

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Introduction: The Western lifestyle greatly contributes to the risk of cardiovascular diseases and Alzheimer's disease. Lifestyle change can improve health prospects dramatically, but executing the change requires firm motivation. Our 18-month lifestyle intervention, "Effects of ApoE4 genotype information and intervention intensity on the fulfilment of lifestyle changes and sensory preferences (ApoE4mot)", assessed the effect of genetic risk information (*APOE ε4* carrier status) on the motivation for lifestyle change.

Materials and Methods: We recruited 211 healthy adult volunteers from Ostrobothnia (Finland), genotyped them for *APOE ε4* carrier status (carrier vs. non-carrier) and communicated the results to the intervention group. The participants filled in questionnaires on lifestyle choices, food preferences and psychological state. Their lifestyle status was documented by physiological measurements and plasma values (cholesterol, blood glucose, fatty acid composition etc.).

Results: 35.6% of the individuals had at least one *ε4* allele. LDL and total cholesterol levels were lower in those with *APOE ε2* than those with *APOE ε3* and *ε4* ($p<0.05$). However, in those with *ε2*, the proportions of 18:2n-6 and total polyunsaturated fatty acids, for example, were lower than in those with *ε4*, and 16:0 and total saturated fatty acids higher ($p<0.05$).

Conclusions: The plasma fatty acid differences may result from genetic differences in the fatty acid metabolism between the *APOE* genotypes, or, excepting the cholesterol values, the slightly better lifestyles of the *APOE ε4* carriers, suggesting better health-awareness. The study population was a representative sample of the Finnish population,

forming a reliable basis for a genetic risk information intervention.

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P20.03C

Communication of Clinical Uncertainties: A Systematic Literature Review

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Uncertainties pervade medicine, and genomics is no exception. How patients perceive uncertainty is influenced by how providers convey it. Communication theories about managing uncertain health information include provider messaging. To assess the state of the science, we conducted a systematic literature review on communication of uncertain health risk information across specialties. Our aims were to assess the breadth and quality of the evidence, identify research gaps and posit evidence-based hypotheses. The search identified 1020 abstracts from PubMed, Web of Science, PsycINFO, Communication Source and Cochrane Reviews published between 1/1/1990 and 6/1/2018. Forty-one met inclusion criteria: 16 quantitative and 25 qualitative studies. A total of 3656 providers and 4530 patients were represented. Among the quantitative studies, one was of high quality--an intervention study to enhance effective communication of uncertainty. Nine were moderate quality observational studies. Seven were self-report of lower quality. Among 25 qualitative studies, 12 were analyses of recorded clinical sessions of high quality, ten were self-report interviews, three were focus groups and one was an ethnographic study. Communication and management of clinical uncertainties were observed or reported between providers and patients in primary care, advanced cancer care, genetics, obstetrics, oncology, cardiology, and emergency medicine. Research gaps exist in intervention research. Evidence revealed providers' avoidance of communicating clinical uncertainties, recognition of challenges in communicating uncertainties, decisions to provide information over uncertainties, and relational factors that led to expressions of uncertainty; all generating hypotheses to be tested. High-quality research is needed to inform best practices in clinical medicine, particularly in genomics.

B.B. Biesecker: A. Employment (full or part-time); Modest; RTI International Non-profit. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; NIH Grant Consultant-Baylor Univ. and Kaiser-Permanente. **B. Boyea:** A. Employment (full or part-time); Modest; RTI International Non-profit. **N. Kulkarni:** A. Employment (full or part-time); Modest; RTI International Non-profit. **H.L. Peay:** A. Employment (full or part-time); Modest; RTI International Non-profit. **R.S. Paquin:** A. Employment (full or part-time); Modest; RTI International Non-profit. **A.C. Wheeler:** A. Employment (full or part-time); Modest; RTI International, Non-profit. **M.A. Lewis:** A. Employment (full or part-time); Modest; RTI International Non-profit.

P20.04D

The concept of 'emotional distress' as a measure of outcome when assessing the impact of genetic test results; a systematic review

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Purpose: In psychological assessment a wide range of terms are used to describe an individual's behaviour, personality and state of wellbeing. Across the genomics research literature there are myriad studies using a plethora of psychological terms, not always consistently. As well as the terminology utilised, the tools employed to measure these states also vary. This review examined the usage and measurement of 'emotional distress' in the context of genetic testing.

Methods: This systematic review followed the Centre for Reviews and Dissemination (CRD) framework and MEDLINE, EMBASE and PsycINFO databases were searched. The Preferred Reporting Items for Systematic Reviews and Meta- Analyses (PRISMA) framework was used to report results followed by a quality scoring analysis. Search terms included "emotional distress", "psychological distress" and "gene*". Qualitative thematic analysis was conducted and tools used within the included studies were quantitatively tabulated.

Results: The review included 13 papers, incorporating 16 tools. Thematic analysis generated six major themes; demographics, influence of patients' condition, influence of time on patient emotion, support structures and mechanisms and familial implications, emotions surrounding genetic testing and methods of emotional measurement.

Conclusion: Terms used when measuring psychological states differed and it is clear that the term 'emotional distress' is being used inconsistently. There is also variation in the application of tools. It is suggested that incorrect assumptions or misunderstanding regarding term usage and psychological health measurement, may result in disparities in patient care. This is of concern when assignment of a particular term is being used to stratify patient follow-up.

S. Eddy: None. **L.M. Jackson:** None.

P20.05A

Patient's experiences of genomic testing for Familial Hypercholesterolaemia: What it might reveal about the adoption of healthy lifestyle behaviours

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Introduction: Recent scientific advances have led to genomic testing being mainstreamed into clinical practice. Consequently, there has been increasing interest in the change in health-related behaviour after patients receive a genomic test result. Leventhal's Self-regulation model is one of the frameworks used in this area. According to this theory, the genomic test result (external stimulus) would change the patient's mental representation about health threats. Recent evidence suggests that simply acquiring new information about one's risk to develop a certain condition might not be enough for patients to change their lifestyle (Hollands et al., 2016). Therefore, we will also be interested to ascertain if taking part in the study might influence the intention to change particular behaviours (Prochaska & DiClemente, 1982).

Our aim is to explore patients' experiences of genomic testing for Familial Hypercholesterolaemia (FH) and understand what factors may explain their readiness to adopt (or not) a healthier lifestyle.

Materials and Methods: We will report a mixed-methods approach that evaluates patients receiving different types of FH genomic test results. Quantitative data will be generated through a questionnaire that includes measures of anxiety, beliefs and perceived control over heart disease and stages of change for smoking cessation and exercise. Qualitative data will be collected through semi-structured interviews and the framework model will be used in the analysis.

Conclusion: Following the example of FH, we will explore how genomic testing in primary care might be used as a potential intervention to empower patients to adopt healthier lifestyle behaviours.

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P20.06B

Psychological and ethical considerations of population genomic testing: qualitative interviews with the public

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Introduction: As genomic technologies rapidly improve, the incorporation of genomic testing into public health prevention strategies and screening programs is being investigated. To assess whether genomic testing should be implemented on a population-scale, and to ensure effective research translation, evidence is required on the psychological and ethical implications of this approach. The perspectives of main stakeholders, such as the public, are key to understanding these implications.

Methods: Semi-structured interviews were undertaken with 30 participants (24-69 years, 50% female) recruited from a pilot trial in which they received personalised melanoma genomic risk information. We explored participants' psychological responses to receiving this information and their views on broader ethical considerations of offering genomic risk information to the population. Data were analysed thematically.

Results: Many participants described positive responses to receiving genomic risk information, including feeling reassured. Some reported short-term negative emotional reactions that dissipated over time. Their responses were linked to: expectations for their risk result, disease risk perception, existing preventive behaviours and their risk results. Participants raised ethical considerations of population-scale genomic testing including: consequences for individual responsibility for disease prevention, possible adverse responses due to perceptions of genetic exceptionalism, implications for parental responsibilities and potential discrimination. Participants felt that trust, choice and equity should be taken into account in the provision of population-scale genomic testing.

Conclusions: Personalised melanoma genomic risk information alongside education and lifestyle counselling is

favourably received by the public with minimal negative responses. Ethical considerations relevant to policy discussion and program design include trust, choice and equity.

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P20.08D

Transgenerational management of information about Huntington disease within the family: from past silence to normalization in the present

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Huntington disease (HD) has a significant impact on family process. Management of the information on HD in families has been scarcely reported, from a transgenerational perspective. This study aims to get a deeper understanding of the management of information about the disease, within Portuguese HD families, and its implications for family and social relationships.

This is an exploratory qualitative study, using a semi-structured interview with persons from HD families. Ten participants were recruited: two carriers, eight non-carriers. Interviews were transcribed and submitted to theme analysis.

Information from genetic diagnosis and the potential for presymptomatic testing represent a turning point for HD family members, with a high impact for management of the information about the disease within the family. After a diagnosis of HD, families tend to identify possibly affected relatives (in previous and present generations) and question who may develop it in the future. Through this process, families i) travel into their past, to re/build the history of the disease in the family; and ii) build a new consciousness about HD and its hereditary character. Family members use different approaches to deal with HD in the family and in the community: either closure (denial, silence, secrecy) or openness (open disclosure). Outcomes from these approaches range from isolation, challenging stigma, and normalizing the disease experience.

These results may be relevant for genetic counselling practice, bringing further insight into transgenerational processes of management of the information about HD in families.

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P20.09A

Noninvasive prenatal test: preferences and awareness among healthcare professionals in Russia

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Introduction: Noninvasive prenatal tests, NIPT, are rapidly changing the algorithm of first trimester screening, FTS, for chromosomal abnormalities, CA. NIPT allows to improve the detection of CA of a fetus and reduce the number of unwarranted invasive procedures. Healthcare professionals, HPs, in Europe are ready to use NIPT as a first or second line test due to its safety, high sensitivity and early results. However, there were found country-specific differences in choosing methods for FTS, depending on ethnic, socio-demographic and religious characteristics of respondents, as well as the local health policy related to the need for partial or full payment of prenatal tests and the availability of abortion. Accordingly, each country requires its own guidelines, developed with taking into account the social context.

Methods: Our group conducted the sociological survey among 37 HPs. The questionnaire included questions about preferences for FTS, demographic attributes and a discrete choice experiment.

Results: For Russian HPs, most significant attributes of prenatal tests are their cost, the justification of invasive procedures and the possibility of obtaining the most

complete information. Don't affect the choice the sensitivity and the time of receiving results.

Conclusion: The obtained results are not fully matched with the data from the world literature, therefore it is necessary to pay attention to the level of awareness of HPs about the possibilities of NIPT, assessment of existing social and ethical problems in the country, as well as the prospects for introducing NIPT into the algorithm of FTS. Grant RFBR №18-013-01175.

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P20.10B
A review of the profile of mood states (POMS) with the client of prenatal test

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Introduction: The availability of prenatal screening and diagnostic testing has changed the experience of pregnancy. Prenatal screening tests may have positive or negative effects for women. In this study, we are looking at the psychological effects of prenatal screening tests for the fetus.

Materials and Methods: A sample of 162 women who had genetic counselling sessions regarding prenatal tests completed the Profile of Mood States (POMS) short form before the prenatal test and after they received the result. The Profile of Mood States (POMS) is a psychological rating scale used to assess transient, distinct mood states such as Tension or Anxiety, Anger or Hostility, Vigor or Activity, Fatigue or Inertia, Depression or Dejection, Confusion or Bewilderment.

Results: Most cases showed slightly higher score in Tension or Anxiety, Confusion or Bewilderment category before the prenatal tests. The score was decreased after they received the test result but some showed repressive and punitive attitudes at the session. Six women showed medically disturbed mood in spite of negative result for NIPT.

Conclusions: Testing the fetus make some impact on pregnant women and the partner even with negative result. The study showed prenatal tests make them slight mood change within expected range in many cases. It was very helpful to find some medically disturbed women through this study since they didn't show any specific symptoms. We take care of the women with positive result, but it might be important to screen all the pregnant women to offer psychological support depend on objective evaluations.

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P20.11C
Transmission of genetic information to relatives in vascular Ehlers-Danlos syndrome

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Vascular Ehlers-Danlos syndrome (vEDS) is a rare inherited disorder leading to arterial, digestive and uterine complications due to pathogenic *COL3A1* variants. Identification of variants allows family screening, provided that relatives have been previously informed accordingly to a 2013 French decree. We wanted to assess the effective transmission of genetic information to relatives and the impact of the diagnosis disclosure, and show a possible link between vEDS experiencing and ability to communicate the genetic information. vEDS adult probands answered a questionnaire during a clinical visit. Transmission of information to relatives was considered effective if the proband gave information to some or all relatives and easily realized if it was done less than a month, without difficulties, to all relatives. Personal and family aspects of probands regarding their vEDS experience were also assessed. Effective transmission of information to relatives was remarkably high (98%). Siblings were the most frequently informed (82%). This process seemed simple to perform (58%) but worrying (44%). Women (89%) seemed to inform their relatives more quickly than men (59%). There was no difference in the information of relatives before and after 2013, meaning that regular multidisciplinary support of patients fosters transmission of information. Regarding vEDS experience, patients felt anxiety (78%) at diagnosis disclosure and considered this diagnosis as the possibility to start a medical follow-up (82%) ; Most of probands with children (56%) felt guilty about vEDS transmission to offspring. The ability to easily communicate information seemed to be mainly related to the relief felt during vEDS diagnosis disclosure.

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P21
Lay beliefs and public understanding of genetics - Access to genetic services

P21.01D
The genome as the most intimate common: taking citizen perspectives into account in policy and research

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Introduction: Genomic research and medicine can only advance as long as there is a willingness to share genomic data. However, given that genomics is both a fringe and highly complex subject, there is a big risk of misinformation, misunderstanding and general distrust in society.

Methods: Different initiatives have been launched to organize the societal debate on ethical, legal and societal issues (ELSI) in genomics. We will focus on in-depth qualitative data from a Belgian citizen forum (32 citizens, 3 weekends) and a French public consultation (22 citizens, 4 weekends) in 2018.

Results: On the one hand, genomic research can benefit everyone and scientific progress is a common good. On the other hand, the individual - and potentially the individual's family - is at risk when certain mutations are discovered or data is misused. In the public's understanding, genes are linked to identity, irreversible traits, the 'nature' of an individual. Both risks and benefits regarding genomics touch an intimate part of individuals' private lives. This tension is not alleviated through societal debate, but discussions between citizens can inform researchers and policy makers about different perspectives in society and potential reactions to certain information, requests, policies and projects. In turn, well organized debates enhance public awareness and understanding of ELSI in genomics. We describe different approaches to societal debates and their outcomes.

Conclusion: Without societal support, the ambitions of genomic research are unattainable. In an area where everyone can feel intimately involved, it is important to actively listen to all perspectives.

W. Van Hoof: None. **C. Mayeur:** None.

P21.02A
Polarised views on the regulation of genetic data and use of genetic information in criminal justice

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The use of genetic data is permeating all areas of society. The talk/poster will present 2 studies dedicated to understanding more about public views on legal applications of genetic data. In the first study, data were collected on how genetic information should be used in criminal trials. Results from the International Genetic Literacy and Attitudes Survey (iGLAS, N=3981) showed marked variation in views. For example, 62% of participants felt that genetic information should inform sentencing, whereas 29% felt that it should not be considered. 6% felt a high genetic risk for aggression should reduce the defendant's sentence; and 3% felt it should increase it. In the second study, participants considered whether family members have a right to information about a close relative's genetic condition (based on ABC vs the NHS). Data were collected several times as additional information was provided to participants. The results revealed highly polarised views. Some participants resolutely supported familial rights to access information about their relative's diagnosis and that the NHS should be legally obliged to provide this. Other participants steadfastly supported the patient's right to confidentiality and non-disclosure, even though this might have health implications for their relatives. Participants who either had a genetic condition themselves or in their family favoured disclosure. Sex differences also emerged. These and other results suggest polarisation of opinions on these important matters and call for increased efforts to involve the public in debates about genetic data usage.

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R. Chapman: None. **V. Smereczynska:** None. **V. Nanau:** None. **D. Matsepuro:** None. **F. Selita:** None. **Y. Kovas:** None.

P21.03B
Opinion about prenatal and gene mutation testing among Polish population

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Aim: The aim of the study was to collect information about knowledge and approach among Polish population to the topic of genetic prenatal tests. We also analyzed the doctor's approaches to the same issues.

Methods: Data collection was carried out by the use of a comprehensive questionnaire on the Internet and in paper format. 2210 people were examined. 204 doctors answered the same set of questions with few addition.

Results: 60,6% of respondents are enthusiastic about prenatal test such as amniocentesis. Over 26% patients and 30,9% doctors consider this test too risky. Free fetal DNA is considered to be very important test by 86,1% of doctors and 75,1% of patients (the respondents were informed about the need of additional payment). Also we asked what is the attitude of respondents to perform genetic testing for BRCA1 / BRCA2 / BRAX without a family history of cancer. 25.9% of respondents do not have any knowledge about such tests and 90.3% of doctors replied that such a study would have a positive impact. Unfortunately doctors do not know how to properly collect samples (82,5%) and what documents to fill in to correctly carry out the procedure (43,3%).

Conclusions: The opinion on genetic testing among Poles is very positive. There is a great need to increase the availability of prenatal and other genetics tests in Poland. It seems very necessary to expand the knowledge of both patients and physicians on subject of genetic research.

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P21.04C
The human germline gene editing dialogue in the Netherlands

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Introduction: Safe and effective clinical application of human germline gene editing (GLGE) is becoming more feasible, as gene editing techniques are being improved. This provides a real prospect of curative treatment for serious (mono)genetic diseases. However, human GLGE may also be used to prevent disease, as in He Jiankui's experiment, and to enhance humanity. There is general consensus that human GLGE should be democratically governed, meaning that all stakeholders should be involved in determining the purposes and boundaries of the technology. Therefore, a public dialogue will be organized in the Netherlands to provide information, encourage active participation, and to assemble various stakeholders' opinions. We aim to investigate the impact of this human GLGE dialogue on public opinions.

Method: This study was piloted among 28 students who filled in a survey on attitudes towards human GLGE before and after a ten weeks minor course.

Results: Student definitions of germline editing were significantly more correct after the minor period compared to before ($p<0.01$). Attitudes towards GLGE shifted equally towards both directions between pre and post measurement. Proponents perceived GLGE to be more promising ($p<.01$) and hopeful ($p<.05$) and judged the odds of the best-case scenario to occur as greater ($p<.01$) compared to opponents.

Conclusions: Increased knowledge does not imply a more positive attitude towards human GLGE. Our data indicates that especially factors related to trust and affect may influence attitudes towards GLGE. Together these findings support the presumption that organizing a public dialogue will enable public deliberation without influencing public opinion.

D. Houtman: None. **S. Riedijk:** None. **B. Vijlbrief:** None. **R. Hofstra:** None.

P21.07B
Most issues covered but not in their full complexity: a newspaper content analysis of ethical issues in predictive genetic testing

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Predictive genetic testing is becoming more available to the general population through direct-to-consumer genetic testing. Its application raises many ethical issues of considerable complexity. Broadsheet newspapers are one of the public's main source of information on such scientific topics. We analysed all articles about predictive genetic testing published in *The Guardian* and the *Daily Telegraph* between 2011 and 2016. Principles of Biomedical Ethics by Beauchamp and Childress and a review of scientific literature served as a starting point for the deductive analysis. The analysis was qualitative with semi-quantitative aspects. We found that issues related to the principles of beneficence and autonomy were covered more than aspects regarding the principles of justice and nonmaleficence. More specifically, the medical benefits of predictive genetic testing were preferred in newspaper coverage over its potential harms. Many ethical issues were

not portrayed in their full complexity, and there was a lack of coverage regarding the autonomy-related issues *Right not to know* and *Incidental findings*. With direct-to-consumer genetic testing on the rise, the ethical issues regarding predictive genetic testing are highly significant for society. Medical professionals, journalists and other stakeholders need to improve the representation of such issues in communication to the public, especially through broadsheet newspapers as the public's main trusted source of information. This study also provides a new methodological approach on how to empirically assess ethical issues in media coverage.

B.M. Zimmermann: None. **B.S. Elger:** None. **D.M. Shaw:** None.

P21.08C

Risk perceptions for Type 2 diabetes and coronary heart disease after receiving risk information - participants of P5 FinHealth study

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Providing risk information to people is expected to change their perceptions of risk and motivate them to change their behaviour. The aim of this presentation is to examine how actual risk estimates correlate with perceived risk and how genetic knowledge is related to these estimates.

Participants of FinHealth 2017 study received a profile based on clinical and self-reported measures. The profile included risk for developing type 2 diabetes (T2D) and risk for developing coronary heart disease (CHD) in the next 10 years. After receiving the health profile, 3384 participants consented to P5 study to receive genetic and metabolomic risk information. Those who consented to study answered questions related to genetic knowledge and perceived risk for CHD and T2D.

At the baseline, 19% of men in age group 50-59 had increased risk (>10%) for CHD and 71% in oldest age group 60-69, respectively among women 2% and 17%. In the youngest age group (30-39) only 4% and at least moderate risk for T2D, while 33% were in that group among 60-69 years old males, respectively among women 11% and 40% had at least moderate risk. Only 10% of all P5 respondents reported their risk to be high for CHD, while 33% reported their risk to be at least moderate for T2D. Genetic knowledge was weakly related to perceived T2D risk (r=-.05,p=.023) and CHD risk (r=-.06,p=.017).

Respondents' perceived risks were strongly related to actual risk estimates. P5 study participants will receive new risk estimates based on polygenic and metabolomic analyses.

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P21.09D

Surveying the general public on genome editing: 10 tips on how to do it to make it count

V. Romano, H. C. Howard

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Surveying the general public on a game-changing technology such as CRISPR-Cas9 is essential for at least two main reasons. First, surveying the general public aims at understanding people's attitudes and orientations about genome editing which are relevant as they should inform, in some ways, further public engagement, scientific research and policy. Second, it simultaneously educates the general public about the surveyed matter and drives people's attention on the subject in a chosen way. Surveying on any subject, no matter how "merely descriptive" the explicit goal of the survey is, always comes as a selection of what has to be considered relevant, why and how. In order for such surveys to not be (too) biased and to increase the knowledge about genome editing, they therefore need to follow a structured design and rigorous methodology.We analyzed the only 8 surveys on genome editing - published worldwide in the last four years and fully dedicated to this subject - and here present the comparative analysis of their main strengths and weaknesses. We noticed that the research design often tends to be identified as the statistical sample selection process and that's why we fear this might weaken the heuristic range of the research itself. We suggest how to structure the research design in order to diminish the risk to get expected results Research grants open call 2017 (Medicine and Health) Swedish Research CouncilType of grant: Research Project GrantProject title: Ethical, legal and social issues of gene editing Project start: 2018-01-01 Project end: 2021-12-31

V. Romano: None. **H.C. Howard:** None.

P22

Ethical issues in genetics

P22.01A

Do international recommendations meet citizens' values and needs regarding genomic information?

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Introduction: We compare some of the main results of a Belgian citizen forum (2018) on the use of genomic information, with related recommendations of international professional societies, such as the European Society of Human Genetics.

Materials and Methods: At the request of the Minister of Public Health, we organized a panel of 32 citizens, selected to ensure maximal diversity. During three weekends, they debated and reflected on the ethical, legal and societal issues surrounding the use of genomic information, supported by experts of different backgrounds.

Results: Among the many needs and opinions expressed by citizens, we focus on one key message. Citizens support genomic data sharing for the common good, which they define as scientific research that improves knowledge (on both prevention and diagnostics) to build a fair society where everyone has an equal opportunity to live healthy. However, their support hinges on three conditions:

1) No genetic discrimination: a person should not be a prisoner of his/her genetic makeup
2) Privacy protection: the genome is an unique and intimate part of the individual that warrants protection
3) Individual control: transparency, traceability and decision-making

As genomics grows more and more important, it will impact the lives of many citizens. However, recommendations of international professional societies are based on expert opinion.

We investigate whether the principles underlying these recommendations are in line with fundamental values in society.

Conclusion: Professional recommendations should be informed by fundamental values and needs of citizens.

C. Mayeur: None.

P22.02B

Challenges of prenatal genetic counselling in two cases of CNV of variable expressivity and incomplete penetrace

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The difficulty of genetic counselling in case of fœtus carrier of microdeletion or microduplication syndrome showing variable expressivity and incomplete penetrance consists in bringing appropriate prognosis about the postnatal development. The prognosis is more uncertain when the CNV is inherited from a healthy parent. Here we present two cases of prenatal diagnosis of 1q21.1 deletion with opposite pregnancy issues. The first case is a second pregnancy of healthy parents. During the second trimester US examination the AVSD was detected. The amniocentesis was performed and array-CGH showed 2,6 Mb deletion including *GJA5* and *GJA8*, inherited by the father. The parents were worried from the uncertain prognosis and the family history of relatives with developmental delay, autism and heart defects. They choose to terminate the pregnancy and applied for preimplantation diagnosis for next pregnancy. The second case is a fifth pregnancy of healthy parents showing some learning difficulties. Two of 3 children had learning difficulties/epilepsy. The second trimester US examination showed VSD and an amniocentesis was performed. The array-CGH showed 1,6 Mb deletion in 1q21.1 including *GJA5* and *GJA8*, inherited from the mother. The parents were not worried and the pregnancy was maintained. A number of ethical questions rise in an everyday clinical practice mainly announcing or no the CNV with uncertain pathogenicity and the place of pregnancy termination and preimplantation diagnosis.

R. Stoeva: None. **E. Henry:** None. **M.T. Cheve:** None. **C. Le Caignec:** None. **D. Martin Coignard:** None.

P22.03C

A survey of public attitudes toward genetic services in Georgia

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Objective: The aim of the present study is to assess knowledge and attitudes of Georgian population toward the genetic testing.

Methods: A total of 491 individuals completed surveys. The data were gathered using a convenience sampling. Eligible participants must have been at least 18 years of age,

Georgian-speaking. Respondents were recruited from the annual scientific festival 2018 held in Tbilisi, Tbilisi book festival 2018 and scientific conferences. Participants were surveyed using a Likert-scale written questionnaire conducted by medical students from Tbilisi State Medical University.

Results: Positive attitude were found toward predictive genetic testing. A majority of participants (77.4%) would like to know whether they at risk of developing diseases. Moreover, about 65% of participant would like to test their newborn child to learn which diseases they may develop in adulthood. Most respondents (71.2%) are agreed that couples planning a pregnancy should have a possibility to have a carrier test. Only 20% of respondents agreed that carrier screening for specific diseases will lead to an inferior image of people with these disorders.

Conclusions: Interest in genetic tests is high and attitudes are largely positive in Georgian population. Such investigation is critical to find out ways to start the discussion between the public, policy makers, and healthcare professionals on these issues.

E. Kvaratskhelia: None. **M. Kvintradze:** None. **D. Chokoshvili:** None. **K. Dzagoevi:** None. **S. Surmava:** None. **E. Abzianidze:** None.

P22.04D

Between burdensome and knowledge - predictive genetic testing in two children from a mother diagnosed with Huntington disease

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Huntington disease (HD) is a late onset, progressive, neurodegenerative autosomal dominant disorder. Given this inheritance pattern, there has been a significant and rather sensible controversy whether predictive genetic testing for minors - in a condition for which there is no disease-modifying treatment - is fit. The numerous ethical issues on both sides of the debate refer not only to the psychological, emotional and social impact of the decision making, but also raise the question regarding the cognitive capacities of the children in their different developmental stages and their right to autonomy. The controversy of testing in minors where there is no medical benefit in the immediate future needs to be challenged once again, given the current perspective of the latest trials that cast a vision upon the possibility of tangible treatments. Given the acknowledgment of this new perspective, the discussion addresses the case of a 39 y.o patient, severely affected, diagnosed with Huntington disease, mother of two boys, aged 12 and 17 that show clinical signs. The purpose of this paper is to explore the positive ramifications and possible negative impacts from the predictive genetic testing for the children, given the real possibility of a treatment.

L. Curteanu: None. **C. Rusu:** None. **C. Afuduloai:** None.

P22.05A

Ethical solicitude in medical genetics as perceived from a genetic counselor's perspective in the tribal-based community of Saudi Arabia

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Introduction: The genetics domain is witnessing great advances in diagnosing and predicting genetic diseases. In a clinical setting, autosomal recessive genetic disorders are frequently observed as a result of the high rate of consanguinity. The advances in genomic technologies in recent years have facilitated new tools for gene discovery in humans. Such approaches have already proven to be beneficial for the identification of the genetic causes of several Mendelian disorders. There is a debate over the ethical dilemmas behind providing families with the genetic test results and incidental findings identified by the whole exome sequencing. Thus, this vast source of information can have a multitude of ethical and social implications both for patients and healthcare providers. In this study we aim to study how families of affected children respond when they receive incidental findings. Also we aim to identify how healthcare professionals working within genetic counseling fields descriptively abide by their role and the information-sharing procedures.

Methods: This study was qualitative conducted at King Faisal Specialist Hospital in Riyadh. It included a total of fourteen parents, and a total of fourteen healthcare providers working with children diagnosed with genetic diseases.

Results and conclusion: In this study six strong themes emerged. This study explored the experiences of parents of children affected with genetic diseases at the time of receiving the incidental findings. It also explored the experiences of the healthcare providers attending these families.

References: Abbort, Madue E. "The Evolution of Modern Medicine." Canadian Medical Association Journal 12.3 (1922): 182

A.A. Qari: None. **A. Kashmeeri:** None. **M. Al-Sayed:** None.

P22.06B
Ethical aspects in genetic research

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Introduction: "Effects of ApoE4 genotype information and intervention intensity on the fulfilment of lifestyle changes and sensory preferences" (ApoE4mot) is a nutrigenetic intervention study. Within its ethics work package, a generally usable questionnaire is being developed to study ethical aspects in genetic studies. Informed consent process is a significant step in the recruitment of participants and is the focus of this questionnaire.

Methods and Materials: Participants were recruited through advertisements from South Ostrobothnia, Finland. A closed-ended ethics questionnaire was developed based on literature and discussions among the research group. One part of the questionnaire assessed genetic knowledge and the other parts probed opinions related to recruitment process including informed consent. Answering options were "agree", "disagree" and "don't know". The questionnaire was filled online. The answers are being analyzed through descriptive statistical methods.

Results: The majority of the participants (250/281) answered the ethics questionnaire. As to informed consent, 91% (228/250) thought that enough information was given, 96% was happy with the way information was given and 99.6% felt that enough time was given to consider participation. Most (98%), felt that they understand what signing the consent means. Although 38% thought that consent should be asked each time their information is used in any study, 54% thought asking once was enough.

Conclusion: Participants regarded the informed consent process as satisfactory. The ethics questionnaire was found to be a useful tool and is now being further developed and validated.

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P22.07C
Demarcating the ethical and legal boundaries of Mitochondrial Replacement Therapy: A literature review analysis

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Mitochondrial Replacement Therapy (MRT) is a new type of *in vitro* fertilization that aims to prevent the transmission of mitochondrial diseases (matrilineal transmission) by replacing mutated mitochondrial DNA in unfertilized oocytes or zygotes with normal mitochondria from a healthy donor. Since as a result of MRT, permanent changes are made to the germline that would be transmitted through generations, this controversially so-called "three parent IVF" can be considered as a means of genetic modification. Besides the UK, which became the first country to approve MRT in 2015, only a few countries have addressed MRT through public policy. In order to explore how the interdependence of ethics, policy, and public views can address questions that arise from new reproductive technologies and analyze the influence of sociocultural factors upon stakeholders' motives for using MRT, we conducted a systematic literature review analysis of relevant academic sources according to Oxford's Bodleian Libraries review guidelines. Next, we reviewed and assessed pertinent legislation, policies, and ethical guidelines, in both the research and clinical contexts, in Canada, the US, the UK, and Mexico whose approaches in governing this controversial technique range from strict bans with serious consequences to an absence of policy. The major themes identified in our review included, safety and efficacy, concerns over germline modification, issues of identity, concerns over three genetic contributors, and distributive justice. Next, the interdisciplinary knowledge produced in the first phase will be used to analyze perceptions and expectations and concerns of Canadian stakeholders with regard to MRT.

F. Noohi: None. **A. Caulfield:** None. **Y. Joly:** None.

P22.08D
Earlier, more and better information in families with a genetic disease

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Introduction: Genetic counselling relies on information circulation within families. One issue of information about genetic risk is reproductive decision making. The aim was to compare attitudes in different neurogenetic diseases such as Huntington disease, spinocerebellar ataxias, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis and Myotonic dystrophy and assess evolution for Huntington disease since 2000.

Materials and Methods: A questionnaire was distributed since June 2018.

Results: We analysed 370 completed questionnaires (200 HD, 81 SCAs, 56 DM1, 28 ALS, 5 CJD). Affected individuals were 34.9%, spouses 27.6%, at-risks persons 21% and premanifest carriers 10.1%, or non-carriers 6.5%. Mean age at response was 56.5± 15.5 (18-85), significantly older in ataxias ($p<0.001$). Only 12.5% knew about the French law, stipulating obligatory information, but 66.6% said they did inform offspring.

37% wished having children, their family disease interfered: 85% declared their disease justified prenatal or preimplantation diagnosis, 67% to interrupt a future pregnancy. Disease gravity estimated by the participant was positively correlated to turn to preimplantation diagnosis ($p<0.01$).

Between 2000 and 2019 participants had been informed about their risk earlier (33.8±13.2 years versus 28.8±14.9 $p<0.05$); they were informed more often by parents, 13.8% versus 51.2%; less by clinicians, 36.6% versus 18.2%; by other family members 20.7% versus 14.0% and not directly, 23.4% versus 16.5% ($p<0.001$) and testing before the age of 18 was considered positively in 13.8% versus 30.1% ($p<0.005$).

Conclusion: Prospective comparison showed that information is given earlier, more frequently and wished to be even earlier for the offspring.

Grant references: Agence de la Biomédecine

J. Hennessy: None. **M. Amador:** None. **M. Monin:** None. **G. Coraelli:** None. **A. Heinzmann:** None. **S. Heide:** None. **E. Schaerer:** None. **M. Spentchian:** None. **A. Herson:** None. **S. Sayah:** None. **E. Petit:** None. **D. Heron:** None. **S. Tezenas du Montcel:** None. **M. Gargiulo:** None. **A. Durr:** None.

P22.11C
searching for secondary findings, considering actionability and preserving the right not to know

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As use of high-throughput sequencing is rapidly growing in the clinical setting, vital questions need to be raised over the most appropriate course of action regarding the active search for disease-causing variants extraneous to the clinical question. Although the American College of Genetics and Genomics recommends a list of these secondary findings be identified in all patients, other professional bodies are not in agreement, a view which we share for several reasons. First, as we discuss, the use of actionability as the criterion for determining whether or not to report secondary findings is problematic, particularly as it is currently lacking a clear, precise and widely agreed upon definition. Second, the question remains as to how beneficial the knowledge of these variants is to individuals' health, particularly given the uncertainty associated with the pathogenicity of some of the variants. We suggest it is critical that reasonable choices concerning the release of data resulting from high-throughput sequencing are made and that it is appropriate to wait for a consensus among scientists on the pathogenicity and clinical utility of disclosing secondary findings before integrating this into sequencing practices in the clinical setting.

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P22.12D
Genetic discrimination concerns in travel insurance - the pre-existing medical condition rule

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Internationally steps have been taken to prevent or limit genetic discrimination in life insurance, yet travel insurance remains unregulated and has received little attention to date. An on-line survey explored the experiences of consumers applying for a range of risk-rated insurance products who had had predictive genetic testing 2010-2016 and asymptomatic at the time of application. Recruitment was conducted through Australian support groups and research organisations. 67/79 respondents at risk for one or more of 76 conditions were valid for analysis. Of the 31/67 who applied for travel insurance after genetic testing, seven [hereditary breast/ovarian cancer - HBOC (4), cardiovascular (2), neuromuscular (1)] reported being charged higher premiums (1) or refusal (5) with the reasons cited as having a pre-existing condition. Another respondent at risk for HBOC reported high anxiety that she had answered "no" to the pre-existing question and may have difficulties claiming if she developed breast cancer. An audit of application forms for travel insurance available in Australia found none included any reference to positive genetic test results as a criterion that meets the definition of a 'Pre-Existing Medical Condition'. While limitations of the survey methodology prevent confirmation of their asymptomatic status, there is the potential that these consumers inferred themselves that they had the condition as a result of their test. This emphasizes the need for clear guidelines for providers of travel insurance, education of consumers as to the meaning of their test result and of the importance of the language used by professionals when returning the result.

K.K. Barlow-Stewart: None. **B.V. Murillo:** None. **M. Otlowski:** None. **A. Doble:** None.

P23
Legal implications of advances in genetics

P23.01A
The GDPR and the research exemption: considerations on the necessary safeguards for research biobanks

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The General Data Protection Regulation (GDPR) came into force in May 2018. The aspiration of providing for a high level of protection to individuals' personal data, risked placing considerable constraints on scientific research, that was contrary to various research traditions across the EU. Therefore, along with the set of carefully outlined data subjects' rights, the GDPR also provides for a two-level framework to enable derogations from these rights when scientific research is concerned. First, by directly invoking provisions of the GDPR on a condition that safeguards that must include 'technical and organisational measures' are in place. Second and in addition to that, through the Member State law. Although these derogations are allowed in the name of scientific research, they can simultaneously be seen as challenging in light of the ethical requirements and long-existed protection standards in biobanking that have been set forth in various research-related soft legal tools, international treaties and other legal instruments. In this paper we review such soft legal tools, international treaties and other legal instruments that regulate the use of health research data. We report on the results of this review, and analyse the rights contained within the GDPR and Article 89 of the GDPR vis-à-vis these instruments. These instruments were also reviewed to provide guidance on possible safeguards that should be followed when implementing any derogations. To conclude, we will offer some commentary on limits of the derogations under the GDPR and appropriate safeguards to ensure compliance with standard ethical requirements.

S. Ciara: None. **S. Slokenberga:** None. **D. Mascalzoni:** None.

P23.02B
Achieving a moratorium on the use of genetic test results in Australian life insurance: a case study

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Introduction: Historically, Australian life insurance companies have been permitted to use genetic test results of any kind in life insurance underwriting, and legally discriminate on that basis. Despite international trends towards banning or restricting this practice, the Australian insurance industry has, until recently, opposed any restriction of access to applicants' genetic results. This has resulted in the deterred uptake of clinical genetic testing and reduced participation in medical research, and has compromised progress in genomic medicine for Australia (Tiller et al, Front. Public Health, 2018).

Methods: In 2016, the Australian Genetic Non-Discrimination Working Group formed to address issues of genetic discrimination in Australia, especially in life insurance. Over the past 2 years, we undertook research, lobbied government, and gave evidence at public hearings for a national Parliamentary Inquiry. We also appeared in the media and surveyed hundreds of Australians with

genetic mutations about their experiences of insurance discrimination.

Results: The survey data we collected indicated a widespread issue, based on consumer-reported breaches of current policy by insurance companies. In early 2018, the Parliamentary Inquiry recommended an urgent ban on using genetic results in life-insurance underwriting. In late 2018, the peak insurance industry body (Financial Services Council) proposed a moratorium to commence in mid-2019.

Conclusion: Achieving a moratorium on the use of genetic test results in Australian life insurance underwriting was a concerted effort requiring the cooperation of numerous groups and individuals and significant time and resource commitments. This case study is highly relevant for all jurisdictions considering these issues.

J.M. Tiller: None. **M. Otlowski:** None. **P. Lacaze:** None.

P23.03C

Improvement of the Russian intellectual property law in order to protect the results of genomic research

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Introduction: The Civil Code of the Russian Federation contains an exhaustive list of intellectual property that is granted legal protection, but it lacks "the results of genomic research". At the same time, the results of genomic studies require legal protection. For the purpose of legal protection of the genomic research results, it is necessary to apply the mechanisms used in the field of intellectual property that meet international standards.

Materials and Methods: The aim of the study was to improve a legal basis for protection of the results of genomic research. A method for analyzing the doctrine, judicial practice, comparative legal methods, as well as the method of legal modeling were used. The specific mechanisms were proposed to protect the results of genomic research.

Results: To protect the results of genomic research in the Russian legal system, the legal regime of copyright, patent law, as well as trade secrets can be applied. In addition, we have defined the limits of applying the legal regime of intellectual property to protect the results of genomic research in the Russian legal system.

Conclusion: The recommendations were made to provide the protection of genomic research results. In particular, the interpretation of Russian legislation on intellectual property objects is carried out in the aspect of protection of the genomic research results. In addition, comments are provided on the decisions of the courts of the Russian Federation on disputes related to genomic research.

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A.A. Inyushkin: None. **V.D. Ruzanova:** None. **E.S. Kryukova:** None. **I.S. Povarov:** None.

P23.04D

Analysis of the procedure for agreement of subjects of the genomic information in the Russian Federation to obtain their biological material for research

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Introduction: Legislation in the field of obtaining the genomic information in the Russian Federation consists of a large number of regulations and does not have a precise system. This leads to inconsistency of legal norms and creates a problem of the agreement for obtaining the biological material for its analyzing, classification, accounting and subsequent use.

Materials and Methods: The genomic information trafficking, as well as judicial practice including the practice of the European Court of Human Rights were analyzed in the study. A method for analyzing the doctrine, judicial practice, comparative legal methods as well as the method of legal modeling were used.

Results: The study suggests the systematization of cases of compulsory and optional coordination of the form, procedure, and conditions in which agreement must be achieved for obtaining biological material for the purpose of subsequent identification of genomic information. A systematic approach to the analysis of the legal system in the Russian Federation has being developed.

Conclusion: The results correlate with legislation and judicial practice in the Russian Federation and in foreign legal systems. The similarities and differences in approaches to obtaining consent to the processing of biological material are revealed. Optimal approaches to the application of the normative base in the field of genomic information collection are developed. The study was funded by RFBR according to the research project № 18-29-14073

V.D. Ruzanova: None. **I.S. Povarov:** None. **E.S. Kryukova:** None. **A.A. Inyushkin:** None.

P23.05A

Patent Protection of the Inventions Created by the Therapeutic Cloning

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Introduction: One of the latest and most controversial issues of biotechnology is therapeutic cloning as it comprises morally and ethically ambiguous question regarding embryo stem cell research. As inventors and scientists tend to protect their intellectual property and resources applying for a patent, opposing debate occurs as to the feasibility to grant patent protection. Notwithstanding the controversy, scientists and inventors have possibility to avail patent protection also for inventions of therapeutic cloning.

Materials: books, journals, reports as well as law and case-law in different languages.

Methods: 1) comparative method; 2) historical method; 3) analytical method; 4) inductive and deductive methods; 5) logical method; 6) grammatical, systemic and teleological methods.

Results: controversy exists as to what interests of an embryo prevail - rights to be born, rights to be born healthy and rights to health or rights to human dignity. The concept of 'being healthy' leaves a margin of interpretation - whether 'healthy' includes generally stated standard in the state or it may comprise the standard which an individual may attain if it exceeds the generally stated level. The debate increases even more in countries which have stated the status of an embryo at a constitutional level. Inventions regarding the therapeutic cloning may be patented in the USA as such, including all types of patents even embryo stem cells. Whereas, amongst the European States may be claimed via micro-biological invention approach, including all types of patents but not involving methods which leads to destruction of human embryo even comprising supernumerary embryos.

L. Rudzite: None.

P24
Other relevant ELPAG issues in genetics

P24.02C
Modelling the economic impact of next generation sequencing on childhood cancer management—a microsimulation approach

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Introduction: Economic cost of cancer care could be improved via the implementation of precision medicine. The aims of this study is to i) determine the costs and hospital utilisation by childhood cancer patient through current practice; and ii) develop a microsimulation model to evaluate the cost and benefit from applying next generation sequencing (NGS) in the management of childhood cancer. The model will be flexible to allow for updating input parameters in future.

Materials and Methods: We used linked datasets of children under 18 year of age, living in New South Wales (NSW), Australia, who have had cancer as base population. Their records were extracted from the NSW Central Cancer Registry and were linked to mortality database and the hospital datasets. Individual patient's hospital services usage was determined from the hospital datasets. The simulation will mainly focus on actionable variants identified, and change in management due to the NGS result. The simulation will be benchmarked against the current evidence, and sensitivity analysis will be performed.

Results: Acute lymphoblastic leukaemia, neuroblastoma, and Hodgkin's lymphoma, accounted for 30% of the reported cases. The annual cost of hospitalisation was $ 114,000. Early diagnosis and treatment before the cancer spread are critical drivers to lower costs and hospital utilisation. In our simulation model, 40% of the patients would be diagnosed with actionable variants.

Conclusions: We have successfully determined the baseline cost of caring for childhood cancer. Our microsimulation model will allow us to simulate the impact of NGS on costs and patients' care.

O. Tan: None. **D. Schofield:** None. **R. Shrestha:** None.

P24.03D
Predictors of colonoscopy uptake: psychosocial and healthcare factors

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Introduction: Colorectal cancer (CRC) is the third most frequent form of cancer worldwide, and approximately one third of cases have a positive family history of CRC or associated cancers. Colonoscopy is one of the most

effective methods of screening for CRC. Uptake of colonoscopy is suboptimal, and many countries lack a national screening programme. Our study aims at identifying and ranking the factors contributing to a positive decision to participate in colonoscopy screening in a sample population at risk, in order to inform psychosocial interventions, such as genetic counselling.

Method: The study included 98 individuals aged over 50 years, recruited from senior centres in Romania. Measures included socio-demographic variables, frequency of colonoscopy, previous recommendations for screening and preventive measures, health literacy and family history of cancer. Receiver Operating Characteristic (ROC) analysis was used to establish the discriminative value for each variable between positive and negative decision for colonoscopy screening. Area Under Curve (AUC) coefficients and effect size parameters were calculated.

Results: 23.5% participants reported previous colonoscopy screening. ROC curve analysis shows that colonoscopy uptake is best discriminated by perceived benefits of screening (AUC=0.711, d=0.787, p=0.001), previous recommendations for screening (AUC=0.689, d= 0.697, p=0.007) and previous recommendations for preventive measures (AUC=0.675, d=0.642, p=0.011).

Conclusions: Results show that recommendations from healthcare professionals towards colonoscopy adherence and preventive measures and highlighting the benefits of screening were associated with improved colonoscopy uptake. These results can further inform genetic counselling sessions by bringing empirical evidence to emphasize screening recommendations in individuals at risk for CRC.

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P24.04A

"It is written in our genes! What people would like to know?" Economic approaches to reveal preferences for genetic testing. Methods and results based on two projects (SEQUAPRE and PRESAGE) in Health Economics

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Introduction: With the rapid spread of genome sequencing in medicine, it's important to know its acceptability for patients and potential users. Which elements of a genetic test are important to them: do they want to know all results, whatever they are? Under what conditions? Health Economics, thought discrete choice experiments (DCEs), allows to reveal and analyze individual and societal preferences for genetic testing. Here, we will present two studies we conducted in France. They illustrate how Health Economics can investigate the value of genetic information and apprehend the diffusion of genomic technologies.

Materials and Methods: SEQUAPRE investigated **individual preferences**. 513 parents of children with rare diseases who would benefit from NGS were surveyed. PRESAGE investigated **societal preferences**. 2501 respondents representative of the French population were surveyed. In each study, DCE was aimed to investigate preferences as regard to different characteristics that can describe a genetic test such as nature and cost of the results. Econometric analysis investigated the presence of preference heterogeneity.

Results: They stressed how genetic information is valued from individual and societal perspectives and the heterogeneities among respondents: the value of uncertain and secondary results, the role of the clinician in the decision making-process, the willingness to pay for the test.

Conclusions: In the context of "Plan Médecine France Génomique 2025", our results show the importance to understand individual and societal preferences to favour the diffusion of the technology.

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P24.05B

Incidental findings in clinical exome sequencing

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In our Genome Diagnostics laboratory, whole exome sequencing has been performed for more than 15,000 index cases in the last 5 years. After analysis of genes associated with the patient's condition (tier 1), analysis of the entire protein coding sequence can be performed. Especially tier 2 has an intrinsic risk to uncover incidental findings (IFs), referring to the unintended identification of (likely)

pathogenic variants unrelated to the initial clinical question, but of possible medical relevance to patients and their families. In total 15 IFs (0.1%) were detected in tier 1, mainly in the gene panel including all OMIM genes. As expected, the risk of IFs in the second tier is higher: 85 IFs were detected in 5,358 analyses (1.6%). For some diseases, this 1.6% chance of an IF is even higher than the chance of a positive diagnosis. Only 58 of these 85 IFs were located in the 59 medically actionable genes reported by the American College of Medical Genetics and Genomics (ACMG), indicating that in our diagnostic setting there is 1.0% chance of finding a pathogenic variant in one of the ACMG genes. This number differs 2.5-fold from the incidence of secondary findings (deliberate analysis of available data) in these genes in our population (incidence 2.6%, Haer-Wigman *et al*. EJHG (2019)). Strikingly, one third of the IFs reported by us are located in genes that are not on the ACMG gene list. These results ask for a revision of international guidelines on the reporting of IFs in clinical care.

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P24.06C
Long-term economic impacts of exome sequencing for suspected monogenic disorders

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Background: Exome and genome sequencing (ES/GS) has high diagnostic and clinical utility in rare genetic disease diagnosis. However, the current health economic evidence base to support widespread adoption and reimbursement is very limited.

Aim: To undertake the first cost-effectiveness analysis of ES for suspected monogenic disorders in comparison with usual diagnostic care, incorporating forecasting of both patient and family outcomes over a 20-year horizon.

Methods: A cohort of 80 infants who underwent ES and usual diagnostic care in parallel were used to model incremental cost and health outcomes (using quality adjusted life years, QALYs) attributable to ES. Three models were developed: (1) outcomes in patients only, (2) outcomes in patients and first-degree relatives as a result of cascade testing, (3) outcomes in patients and first-degree relatives including parental reproductive outcomes.

Results: When the directly observed cost and health outcomes of the cohort participants were forecast over 20 years, the use of ES resulted in a total gain of 7.63 QALYs for the cohort and an incremental cost-effectiveness ratio (ICER) of AUD$24,887.80 per additional QALY gained. When cascade testing in first-degree relatives was added to the model, cost-effectiveness was substantially increased, generating a total gain of 16.15 QALYs and an ICER of AUD$12,331.17. When parental reproductive outcomes were added to the model, this produced the most cost-effective outcome, with a total QALY gain of 40.55 and an ICER of AUD$11,899.69.

Discussion and Conclusions: ES in suspected monogenic disorders becomes more cost effective as the benefits of cascade testing and reproductive outcomes are realised.

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P24.07D
The role of personality and price on interest in direct-to-consumer genetic testing in a UK sample

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Introduction: The accuracy and accessibility of direct-to-consumer genetic tests (DTCGT) has increased rapidly in the last few years. Consequently, it is important to research who is using these tests and why. This study explored the underlying psychosocial factors that impact on interest in DTCGT, including the influences of personality and price (£149 versus free-of-charge) on the decision-making process.

Methods: Participants (N=168, average age=18-25 years), recruited via social media and email, completed an online survey including the 44-item Big-Five Personality Inventory. This measured: Neuroticism; Extraversion; Conscientiousness; Openness; Agreeableness. Additional questions measured levels of interest in DTCGT and how these vary as a function of price.

Results: Increased conscientiousness was associated with decreased interest in free-of-charge DTCGT, perhaps a result of the perception that genetic tests pose a greater threat to mental health than a benefit to physical health. In participants over 25 years, interest in £149 DTCGT was associated with increased openness. Extraversion was associated with decreased interest in free DTCGT in 18-25

year olds. Neuroticism and agreeableness did not predict interest. 86% of participants asserted a GP recommendation would heighten their interest.

Conclusion: Personality and price both affected interest in DTCGT. Interest peaked when tests were free-of-charge and with a GP recommendation. This suggests the UK's use of genomic medicine will attract significant interest, but patients with different personality traits may require personalised support during the consent process. We are currently exploring the UK public's awareness and acceptance of genomic medicine to further inform NHS consent procedures.

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