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MEETING ABSTRACTS

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E-P01 Reproductive Genetics/Prenatal Genetics

E-P01.01
Parent of origin in familial 22q11.2 deletions impacts full scale intelligence quotient scores

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Background: Familial 22q11.2 deletions are thought to have a negative impact on mean Full Scale IQ scores (MFSIQ) in affected offspring, however, parent of origin (PO) effect has not been examined.

Methods: We compared MFSIQ on children of affected parents from Philadelphia (N=26) and Leuven (N=26), assessed using the age appropriate Wechsler Intelligence Scale, in those with familial v. *de novo* deletions and maternally v. paternally inherited familial deletions. We then compared this data to *de novo* cases where research based PO studies were completed (N=57) in NY.

Results: MFSIQ (66.4) for familial deletions was statistically lower ($p = .01$) than for *de novo* deletions (N=399, MFSIQ=76.2). MFSIQ for children with maternally inherited deletions (63.7) was statistically lower ($p = .03$) than for paternally inherited deletions (72.0). As compared with the NY cohort where the MFSIQ for maternal deletions (N=37, MFSIQ=73.41) was no different ($p = 0.67$) than paternal deletions (N=20, MFSIQ=75.2).

Conclusions: Our findings confirm the association of lower FSIQ scores in familial versus *de novo* 22q11.2 deletions. We also observed the novel association of lower MFSIQ scores in maternally v. paternally inherited familial deletions in contrast to *de novo* deletions where no difference was observed based on PO. Thus, a maternally inherited familial deletion is a significant risk factor for poorer cognitive outcome. Confounding factors could include maternal comorbidities, mitochondrial effects, epigenetics, assortative mating, socioeconomics, grandparental engagement, etc. to explain this finding. Regardless, this data serves as an important adjunct to traditional genetic counseling for women with 22q11.2DS in the prenatal and preconception setting.

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E-P01.02

Reevaluation of ADAM12 as efficient marker for X chromosome trisomy prenatal biochemical screening for improving care of associated clinically important disorders of postnatal development

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Introduction: ADAM12 assures bioavailibility of IGF-1/IGF-2 for growth, differentiation and neurogenesis and is expressed in the trophoblast. Trisomy X (TX) is the most common chromosomal abnormality (est. 1:1000 females) with a variable a phenotype, commonly associated with renal/urogenital abnormalities, developmental delay, depression, autism, schizophrenia and cerebral cortex hypo-/hyperplasia. In this regard, we have retrospectively validated this biomarker in prenatal aneuploidy screening in a representative Czech cohort.

Material and Methods: ADAM12-S serum levels were measured in 1534 frozen maternal sera by Delfia ADAM12 research kit (Perkin Elmer) using time-resolved fluoroimmunoassay (within 9th-18th weeks of gestation) at percentile level distribution <5, 6-10, 11-25, 25-49, 50, 51-75 and 76-100. We assessed screening efficacy of autosomal (70) and heterochromosomal (30) aneuploidies.

Results and discussion: Levels of ADAM12 in all percentile categories were increasing by 15 ng/ml/day ($p<0.000001$). Detection rates in category <25th percentile for T21 was 46.67% (45 cases), for T18 57.14% (7), T13 100% (7), triploidy 100% (10), TX 87% (8), monosomy X 12.5% (8) and monosomy X mosaic 16.66% (6).

Conclusions: Although ADAM12 does not satisfactorily detect T21, it is sensitive for the detection of T13, triploidy and TX. Decreased ADAM12 levels could be due to defective X chromosome inactivation, as independently suggested by XIST overexpression associated with psychiatric disorders and TX. Significantly decreased ADAM12 levels are associated with placental dysfunction and impaired CNS differentiation. Only ADAM12 enables prenatal screening of developmental and psychiatric disorders related to TX, and fosters their early prevention and eventually also therapy.

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E-P01.03

Cytogenetic abnormalities in amniocytes and fibroblasts of abortion material diagnosed in the Laboratory of Medical Genetics - Varna for a 10 year period

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Introduction: Amniocentesis is the most widely used method of prenatal cytogenetic diagnosis for its high sensitivity though the risk from the manipulation. Data show that 50% of all clinically recognized pregnancies are due to aneuploidy and 5% - structural chromosomal abnormalities (SCA).

Materials and Methods: Cytogenetic study was performed in 721 samples of amniotic fluid and 19 from fibroblasts. Karyotype was analyzed on GTG-banded metaphases on cultures of amniocytes and fibroblasts, according to the standard protocol.

Results: Overall abnormality rate in amniocytes was 4,32%, mainly with high maternal biochemical risk. These were trisomy 21 (2,2%), trisomy 18 (0,6%), -chromosome numerical and SCA 3 (0,4%); 1 (0,14%) with unbalanced karyotype; 1 (0,14%) - a carrier of two different translocations. SCA were identified in 5 (0,7%) cases indicated for familial rearrangements: 2 balanced translocations, 2 inversions and 1 derivative chromosome, due to three way translocation in the father. Ultrasound data on aneuploidy enabled the detection of 1 fetus (0,14%) with combined numerical and structural karyotype of paternal origin.

The pathology In the fibroblasts was 36,8%: trisomy 18, mosaic translocation 21, one trisomy 11, 13 and 16 and mosaic trisomy 2.

Conclusion: Nowadays amniocentesis is still a feasible tool for detecting structural chromosomal aberrations in 1,4%, which NIPT would fail to diagnose. Analyzing the results of amniocentesis helps us to determine prenatal detection rate of chromosomal aberrations and proper genetic counseling of pregnant women. The pathology found in this study of fibroblasts confirms that most of the early abortions are due to aneuploidy.

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E-P01.04

Molecular dissection of fetal anomalies by using array CGH analysis - the opportunities and challenges

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Background: The systematic review of the literature for the use of array CGH in prenatal diagnostics provides with evidence for the significantly higher sensitivity of this method in the elucidation of high risk pregnancies, especially for structural fetal anomalies. In this study, we evaluated the diagnostic rate of array CGH analysis in cases of disturbed fetal development and high reproductive risk in the family.

Materials and Methods: We applied G4449 SurePrint G3 Hmn CGH 4x44K Oligo microarray (Agilent Technologies) with an average resolution of 150 Kbp, in 30 fetal samples with the mentioned indications.

Results: In 14 samples (47%) we detected pathogenic structural genomic aberrations: deletions of 7q21.3, 10q26.3, 22q11.21, 1p36.22, 4p16.3, 17p13.3, 6q27, 1q21.1, 15q11.2; duplications of 18p, 22q11.1, 22q11.21 or chromothripsis. All deletions/duplications were associated with known disease syndromes. In 8 of the cases (27%) variants of unknown significance (VOUS) were detected: deletion of 17q21.31 and duplications of 18p11.23, 22q12.3, 6p25.1, 11p15.5, 16p13.3, 1q12, 9q34.3.

Conclusion: The array CGH diagnostics in prenatal settings is quite challenging and requires much more focused result, based on the data on known pathogenic aberrations and syndromes; our results showed deletions as the most prevalent pathogenic aberrations. It is extremely important to collect data for VOUS and match them to specific fetal markers, in order to make the feasible use of Fetal-Genomic database.

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E-P01.06

Genetic variation in *CHRNA7* and *CHRFAM7A* is associated with nicotine dependence and response to varenicline treatment

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Introduction: The role of nicotinic acetylcholine receptors (nAChR) in nicotine dependence (ND) is well established; *CHRNA7*, encoding the α7 subunit, has a still uncertain role in ND, although it is implicated in a wide range of neuropsychiatric conditions. *CHRFAM7A*, a hybrid gene containing a partial duplication of *CHRNA7*, has been shown to modulate α7 nAChR function. The aim of this study was to investigate the role of *CHRNA7* and *CHRFAM7A* genetic variants in ND and to test the hypothesis that α7 nAChR variation may modulate the efficacy of varenicline treatment in smoking cessation.

Methods: We assessed *CHRNA7* and *CHRFAM7A* copy number, *CHRFAM7A* exon 6 Δ2bp polymorphism, and sequence variants in the *CHRNA7* proximal promoter in an Italian sample of 408 treatment-seeking smokers. We conducted case-control and quantitative association analyses using two smoking measures (cigarettes per day, CPD, and Fagerström Test for Nicotine Dependence, FTND). Next, driven by the hypothesis that varenicline may exert some of its therapeutic effects through activation of α7 nAChRs, we restricted the analysis to a subgroup of 142 smokers who received varenicline treatment.

Results: The *CHRNA7* promoter variant rs28531779 showed association with both smoking quantitative measures (FNTD $p = 0.027$, CPD $p = 0.012$). Moreover, in the varenicline-treated subgroup we observed association of *CHRFAM7A* copy number with six months smoking abstinence ($p = 0.035$).

Conclusions: Our study points to a possible role of genetic variation in *CHRNA7* and *CHRFAM7A* in tobacco addiction mechanisms and response to varenicline treatment.

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E-P01.07

The cytogenetic study in male infertility

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Male infertility is one of the most simple examples of complex disorder with a defined genetic background. Genetic factors show about 30% of cases of male infertility associated with oligosospermia and azoospermia.

The Purpose: was to study the pecularities of cytogenetic polymorphism in male infertility in order to confirm the importance of cytogenetic diagnosis prior to assisted reproductive techniques (ART).

Material and Methods: A group of 55 infertile men were investigated during genetic counseling in the Center for Reproductive Health and Medical Genetics between 2015 and 2017, having the following selection criteria: more than one year infertility in the couple and / or sperm analysis: azoospermia and severe oligospermia. Karyotyping was performed on peripheral blood lymphocytes according to standard methods G.

Results: Chromosome abnormality has been detected in 8 men with infertile problems.The most common chromosome disorder diagnosed with Klinefelter syndrome was homogeneous form or trisomy 47, XXY (4 cases), followed by mosaic form (47 XXY/46, XY: 1 case), and variants of structural abnormalities of autosomal chromosomes (47, XXY, inv (5): 1 case). One patient was diagnosed with 46,X del (Y)(q1123-qter) and another patient with 46XY,t(8;7)(8qter::7q336-qter).

Conclusions: In the context of the introduction of assisted reproduction techniques, the male partner's cytogenetic evaluation is necessary for diagnosis of male infertility, treatment and provides genetic counseling, including all information on the individual type of chromosome anomaly/polymorphism, its clinical relevance, possible inheritance, prenatal diagnostics.

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E-P01.08

Fetus with partial trisomy 4 and t(2;16) due to maternal complex rearrangement involving three chromosomes: a case report

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Introduction: Complex chromosome rearrangements (CCRs) are structural chromosome anomalies involving two or more chromosomes and more than two breakpoints. These can be balanced rearrangements (BR) which are not causing a chromosomal loss/gain or unbalanced rearrangements (UR) that result in loss/gain of genes at the breakpoints. These gene changes can cause fetal anomalies. It is important that URs can be *de novo* or occurring because of BRs of parents. Here we report a fetus with partial trisomy 4 and t(2;16) due to maternal complex rearrangement.

Clinical Report: A couple, a 22-year-old female and a 32-year-old male, both in good health, was referred for genetic counselling following an abnormal fetal sonogram. This was the couple's second pregnancy following one first trimester miscarriage. They were non consanguineous parents. In the family history, mother's parents had an ex-preterm infant. An ultrasound study performed at 26 weeks' gestation revealed craniosynostosis and anencephaly. At this time, the patient was counselled about the high risk of a fetal chromosome abnormality. Karyotype and multiprobe FISH analyse of the cord blood sample was performed and 47,XY,+der(4)del(4)(q13.2?).ish t(2;16)(q33;q22)(wcp16+;wcp2+),add(4) (?;q13.2?)(wcp4+) was detected. Parental karyotyping revealed a chromosomally normal father and the mother was carrying 46,XX,t(2;16) (q33;q22),ins(2;4)(q31?;q21qter?).ish t(2;16)(q33;q22)(wcp16+;wcp2+),ins(2;4)(q31?;q21?)(wcp4+).
The pregnancy was terminated.

Conclusion: Although CCRs are rare, they are often associated with multiple congenital abnormalities, recurrent spontaneous abortions and infertility. Therefore, genetic counselling for CCR carriers is very important and can be offered before and after pregnancy. References:McGowan-Jordan J, Simons A, Schmid M. ISCN 2016.

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E-P01.10

Detecting a triploid embryo while doing PGD for a cystic fibrosis carrier woman

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Introduction: Preimplantation genetic diagnosis (PGD) is an option for couples with a risk of transmitting a genetic disease, to prevent birth of children affected with monogenic disorders. Other usages of PGD are sex selection and HLA typing. Chromosomal aneuploidies are also detectable in molecular PGD.

Materials and Methods: A family with a 14 year's old child affected with cystic fibrosis referred to our laboratory. Blood samples collected and DNA was extracted. Mutation detection carried out using Sanger sequencing. Linkage analysis performed to trace defective alleles using multiplex short tandem repeats (STRs). Aneuploidy STR markers were checked for the family to illustrate each person's profile. Fertilization carried out at in vitro fertilization (IVF) clinic. After 3 days one blastomere removed from each embryo. Causative mutation and informative STR markers were investigated for each blastomere using multiplex nested PCR. Linkage analysis performed and intended embryos were selected and implanted to mother's uterus.

Results: CFTR: delF508 was detected in patient. From 7 analyzed blastomeres 5 were unaffected. Haplotype mapping illustrated that one of the unaffected blastomeres had an extra set of chromosomes inherited from her mother which was detectable in chromosomes 7, 13, 18, 21 and X.

Conclusions: PGD is a powerful diagnostic tool for carrier couples who desire a healthy child and wish to avoid medical abortion. It can also be used to detect chromosome numerical abnormalities before pregnancy. Results obtained from linkage analysis and haplotype mapping in parallel with direct mutation detection make this method more accurate and reliable.

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E-P01.11
Different results from examination of CVS and amniotic fluid

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We present a pregnancy with different results of CVS and amniotic fluid examination. A 27-year-old woman was referred for genetic counseling because of positive first-trimester screening. Her previous pregnancy ended because of GEU. Prenatal diagnosis was recommended and mother underwent CVS at 13 weeks of gestation. QF PCR from CVS for aneuploidy of chromosomes 13, 18 and 21 was suspicious for trisomy 18, but centromeric FISH revealed chromosome 18 disomy. Ultrasound screening of the fetus didn´t show any abnormalities. Cytogenetic analysis showed karyotype 46,XY,i(18)(q10)[8]/46,XY[2]. This result was confirmed with FISH and QF PCR examination. It was recommended to verify the result from amniotic fluid. Amniocentesis was performed in the 17th week of pregnancy. The karyotype in cultured amniotic fluid cells appeared normal, whereas Array CGH examination identified a 5,4-Mb terminal deletion of chr.18p. Parental karyotypes were normal. The mother was informed about the result: de Grouchy syndrome. The mother was informed about the possibility of pregnancy termination. She decided to continue with the pregnancy. The boy was delivered prematurely in 34 weeks of gestation with birth weight 1940g. Dysmorphic features at birth were mild. The boy at six months of age had weight 7010g, round face, thin hair, broad base to nose, palpebral fissure upslanted, large ears, short neck, inguinal hernia. MRI of the brain was normal. Cytogenetic analysis from peripheral blood lymphocytes showed pathological karyotype 46,XY,del(18)(p11.31), Array CGH confirmed the result. Up to now the development of the boy continues to be favourable, but only time will tell.

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E-P01.12
Rapid, efficient, non-invasive fetal sex determination direct from maternal plasma

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Objective: Many European countries determine fetal sex by analysis of cell free DNA (cfDNA) extracted from maternal plasma, significantly reducing the invasive testing rate for pregnancies at high risk of X-linked disorders or congenital adrenal hyperplasia. Here we present validation of a method analysing unextracted cfDNA direct from maternal plasma in a diagnostic lab.

Method: Our current protocol involves duplicate cfDNA extractions (QIAsymphony SP) from 2ml of double spun

plasma followed by detection of the SRY and CCR5 genes by quantitative real-time PCR (Q/RT-PCR) (Taqman, ABI 7300). We identified 100 stored plasma samples from high risk pregnancies of 7-10 weeks gestation with fetal sex tested previously and clinically confirmed. Up to thirteen 80ul plasma samples with controls were tested per Cell3™Direct: Fetal Sex Determination kit (Nonacus, UK) which detects the SRY, TSPY, DAZ and CCR5 genes by multiplexed Q/RT-PCR.

Results: Thresholds for classification of male or female were established. 89 of the 100 plasmas analysed showed conclusive concordant results first time. This includes 10 of the 13 previously reported inconclusive by our standard protocol and 5 samples below 9 weeks gestation. Of the remaining 11 plasmas, these showed concordance with repeat testing.

Conclusion: This first direct from plasma, non-invasive prenatal test to determine fetal sex is more sensitive than the standard protocol. Removal of the extraction step reduces time, consumables and labour costs. The reduced plasma volume requirement allows for a smaller blood collection or for further testing if required. Ongoing validation will compare the methods in parallel.

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E-P01.13

The relationship between genes related folate metabolism and blood coagulation parameters in Ukrainian couples with reproductive disorders

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Introduction: Folate metabolism disturbance (hyperhomocysteinemia, folate deficiency or excess) and adverse folate genetic variants remain significant risk factors for reproductive disorders. Preventive folate consumption by couples, pregnant women and anticoagulants treatment in clinical cases provided by many national guidelines. But the relationship between these components (when genetically determined thrombophilic disturbances started) in couples with reproductive disorders has not been studied enough.

Materials and Methods: 170 couples (female/male) with reproductive disorders (primary infertility/early pregnancy loss) examined. There were investigated folate related genes polymorphism: *MTHFR* (C677T; A1298C), *MTRR* (A66G), *MTR1* (A2756G); folate metabolism (plasma homocysteine/serum folate levels) and coagulation parameters (activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), fibrinogen level). We collected data about folic acid consumption and life style (including smoking, diet, coffee, physical activity). Statistical analyses done used Pearson's correlation and binary logistic regression (SPSS_17.00).

Results: Plasma homocysteine levels significantly correlated only with serum folate levels of investigated patients. Serum folate levels significantly correlated with additional folate consumption and coagulation parameters (APTT, PT, TT and fibrinogen levels). Folate deficiency (according WHO recommendation 2012) significantly associated with *MTHFR* (1298CC genotype), *MTR1* (2756AG and 2756GG genotypes) genes, family history of cardiovascular diseases and smoking. Folate excess significantly associated with *MTRR* gene (66AA genotype). Fibrinogen levels significantly depended from *MTHFR* (C677T) and *MTR1* (A2756G) genotypes.

Conclusions: Individual rational vitamins use and optimizing folate serum levels can improve in patients with reproductive disorders the quality of infertility treatment and reduce the risk of reproductive losses due coagulation parameters to normalize.

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E-P01.14

The patient with fragile site on chromosome 16 and four missed abortions : a case report

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Introduction: We report a case of 31-year-old woman who had four missed abortions. In first two missed abortions genetic analysis from aborted material was not done, but in third one karyotypic analysis revealed trisomy of chromosome 16 (20 mitosis). In fourth missed abortion the same karyotypic abnormality was detected again, with the fragile site on the long arm of two chromosomes 16 (fra(16)(q22) [10]). Genetic counceling of the couple confirmed no previous missed abortion in their families.

Material and Methods: After analysis of constitutional karyotypes of the partners, we found abnormalities of chromosome 16 in the karyotype of the woman: deletion of 16q22 was found in two mitosis while fragile site of one of the chromosome 16 was seen in 5 mitoses. The rest of 42 mitoses analysed were normal. The karyotype was described as follow: 46,XX,del(16)(q22)[3]/46,XX,fra(16)(q22)[5]/46,XX[42]. Fluorescent *in situ* hybridization (FISH) method with the probe for 16q22 (Abbott, Vysis, CBFB BreakApart probe) showed deletion of the 5' region of the *CBFB* gene in all of the nuclei/metaphases analysed . In addition, array-CGH analysis performed in the woman showed no aberration in 16q22 region, while the next generation sequencing analysis has not been started yet.

Conclusion: To exclude any possibility of trisomy of chromosome 16 in further pregnancies, we suggested *in vitro* fertilization (IVF) followed by preimplantation genetic screening of embryos (PGD). The fragile site of 16q is considered to be a normal variation in human population, but its role in possible missed abortions still have to be completely clarified.

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E-P01.15

Novel serum-free culture conditions for growing primary human granulosa cells

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During folliculogenesis the oocyte is surrounded by granulosa cells (GCs), that are crucial to its development and maturation. The oocyte is not capable of some metabolic processes and needs nutritional support from granulosa cells. They provide the oocyte with growth factors, hormones, and cytokines. Long-term culturing and testing of primary granulosa cells would help to reveal granulosa-related fertility problems. There is still need for more efficient and defined conditions for studying these cells. Our aim was to develop serum-free culture conditions which would support long-term cultivation of primary granulosa cells.

Primary luteinized human GCs were cultivated in serum-free growth media containing either no growth factors, IGF2, FGF2, or both, up to 24 days. The expression levels of GC markers were analysed by qPCR.

The expression of *FSHR* increases while AMHR level does not significantly change during the first four days of culture but start to decrease after that. The expression of *LHR* and *CYP19A1* decreases during time of cell culture. *CYP19A1* is expressed very highly at the starting point but starts decreasing rapidly in cell culture. After four days the expression drops about 2-5 folds.

Currently, this cell culture protocol could be used for short term GC culturing. For long term experiments adding additional hormones like FSH is currently being tested to see if that might help to maintain GC identity.

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Prenatal diagnosis of hypospadias and issues

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Introduction: Urinary tract abnormalities are frequently detected during obstetrical ultrasonography. However, hypospadias is often missed on prenatal US, despite it being the most common congenital defect of the male external genitalia. The prenatal recognition of hypospadias is important because it will alert the physician to order karyotyping and to look for any possible associated dysmorphic syndromes.

Materials and Methods: 16 patients neonatally diagnosed hypospadias between 2007 and 2017 were analysed retrospectively based on obstetrics/neonatal chart and ultrasound data.

Results: Regarding the type of hypospadias, 15/16 were distal hypospadias and only one case was diagnosed as mid-shaft hypospadias. We did not have severe type of hypospadias during 10 years. In total, 8/16 (50%) cases were diagnosed prenatally with ultrasound examination. All the case prenatally diagnosed had other findings such as FGR, CHD. There were two cases diagnosed as syndromic condition neonatally such as ATR-X syndrome and Noonan syndrome and three other syndromic conditions were still on the genomic analysis.

Conclusion: Some reported that severe type of hypospadias could be easier to pick up at prenatal ultrasound examination, however out prenatally diagnosed case were all mild type. It is most important that we have to be aware that those patients might have other complicated conditions

and look carefully as neonates as well. In Japan, most of pregnant women want to know the fetus' s sex, however prenatal ultrasound scans should include a study of the genitals and should not only be used for sex determination.

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Evaluation of Clinical, Cytogenetic and Molecular Parameters in SRY-positive 46,XX Testicular Disorder of Sex Development: Presentation of Nine Cases

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Introduction: 46,XX testicular disorder of sex development (tDSD) is a rare condition with an incidence of 1/20,000 male newborn. Patients have 46,XX karyotype, yet they have male external genitalia and male identity. They have hypogonadism, gynecomastia and azoospermia. Majority of patients have SRY gene translocated to Xp or an autosome. Up till now more than 250 patients have been reported. Here we present 9 more cases.

Methods: Clinical and laboratory findings (hormone profile, sperm analyses) cytogenetics, FISH (SRY-CEPX) and Y microdeletion analysis were studied; genetic results were correlated with clinical and laboratory parameters.

Results: Mean height was below the mean height of normal Turkish men (166.7 cm versus 174.3 cm); mean age and weight was normal. All cases had a decreased testicular volume, all had male phenotype. All patients had hypergonadotropic hypogonadism and azoospermia. 6/9 patients had a decreased pubic and axillary hair. 3/9 patients had gynecomastia. 6 of 9 patients had 46,XX, 3 patients had Y to X translocation. In FISH analysis SRY gene have been located on Xp. Y microdeletion analysis revealed that AZFa, AZFb, AZFc and AZFd regions were deleted but SRY was present in all patients.

Conclusion: All of our patients had a SRY component. All had azoospermia, gonadotropine levels were high in all of them. All cases presented with male external genitalia and hypogonadism in contrast to SRY(-) tDSD which presents mostly with ambiguous genitalia. Azoospermia was suggested to be the result of AZFa,b,c and d region deletions.

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E-P01.19

A rare case in literature: isochromosome Xq in Klinefelter syndrome

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Introduction: Klinefelter syndrome(KS), affecting 1 in 500 to 1,000 newborn males, is the most common sex chromosome aneuploidy among males with primary hypogonadism. Isochromosome Xq on the other hand, is a rare variant of Klinefelter syndrome, accounting approximately 0.3% of all KS and associated with normal height and androgenization compared to classical KS. Here we present another case of isochromosome Xq variant of KS with similar clinical and cytogenetic findings with the few cases reported before.

Materials and Methods: 25 years old male patient referred to our clinic with complaint of infertility. He is son of a consanguineous couple who are first cousins and there was no family history of reproductive difficulty. In physical examination synophrys, prominent ear and small testicles noted. His height, weight and secondary sexual characteristics were within normal range. The patient's spermiogram showed azoospermia and scrotal USG revealed testicular atrophy with heterogenity in paranchyma.

Results: Karyotype analysis using G-banding resulted as 47,X,i(X)(q10),Y and Short Tandem Repeat (STR) analysis showed no deletion in AZF and SRY loci of interest.

Conclusion: Although dozens of isochromosome Xq variant of KS can be found in literature, it is our duty to expand our knowledge about this syndrome as far as possible and emphasize importance of karyotyping for patients with reproductive diffuculty who may not have all features of a well-known syndrome.

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E-P01.20

***MAGEL2* in the prenatal setting: Beyond fetal akinesia and arthrogryposis**

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Introduction: Truncating mutation on the paternal allele of *MAGEL2* is associated with Schaaf-Yang syndrome. The phenotype of affected individuals ranges from lethal arthrogryposis multiplex to mild intellectual disability or autism spectrum disorder. Approximately 30 cases have been reported to date, but data on prenatal phenotype are scarce.

Methods: We performed a file review of six patients (including three from the same family, and two siblings) diagnosed after birth with Schaaf-Yang syndrome at our centers in a clinical setting, compiled prenatal and molecular data, and reviewed the literature.

Results: Prenatal findings in our patients include fetal akinesia (2/6), pyelectasis (2/6), increased nuchal translucency (1/4), intra-uterine growth retardation (1/6), contractures (1/6), and cerebral anomalies (1/6). All were severely affected after birth with progressive contractures and developmental delay. Two had severe hydronephrosis, while another had renal failure. Molecular analyses identified truncating variants in *MAGEL2*: c.1762C>T, c.3043C>T, and c.1996dupC. We reviewed data on 33 cases reported between 2009 and 2017, including 10 with prenatal manifestations. Prenatal features include fetal akinesia (7/10), contractures (4/10), and polyhydramnios (3/10). In addition, two children without reported prenatal features were small for gestational age.

Conclusions: Although Schaaf-Yang syndrome can present prenatally, the features may be mild or absent. Fetal akinesia, contractures, and polyhydramnios are recurrent, but are absent in more than 50% of cases. The syndrome should be considered when fetal akinesia or contractures are associated with growth retardation, pyelectasis or oligohydramnios.

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E-P01.24
Array CGH usefulness identifying a low-level mosaic small marker chromosome detected at prenatal diagnosis

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Introduction: Detection of a mosaic small marker chromosome (SMC) represents a diagnostic challenge for prenatal diagnosis. Their clinical relevance depends on factors such as size, genetic content, and distribution of mosaicism. Herein, we present a case of low-level mosaic SMC characterized by arrayCGH allowing to evaluate and discard UPD, and to perform a more accurate genetic counselling.

Patient and Results: A 38 years old woman was referred for prenatal diagnosis because of maternal anxiety. Karyotype of amniotic fluid cells showed a "de novo" SMC in 20% of metaphases analyzed, from two independent culture flasks. C-band staining was performed; the results suggested that the small marker chromosome was not composed only of heterochromatin. The CytoSure™ Constitutional v3 8x60k (Oxford Gene Technology, UK) was used according to the manufacturer's protocol. The sample was analyzed as a patient once and as a control twice in order to distinguish between a real copy number variation from an artifact. The results were compatible with a 5.4 Mb dose increase from the 7p12.1p11.2 band. Fluorescence "in situ" hybridization (FISH) with a centromeric chromosome 7 probe was performed. Three hybridization signals were detected in 9.5% of the nuclei analyzed and the origin of the marker chromosome was confirmed as derived from chromosome 7. UPD(7) was excluded.

Conclusions: - ArrayCGH is a powerful tool to characterize SMCs regarding size and genes even in low-level mosaics.

- The appropriate use of conventional and molecular cytogenetic techniques can be determinant for more accurate SMC/phenotype correlation improving the genetic counselling.

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E-P01.25
Minor ultrasonographic findings with major fetal chromosomal abnormalities in prenatal diagnosis

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Introduction: Sonographic markers have been shown to be effective in screening for aneuploid conditions; moreover, ultrasonography is a non-invasive, risk-free method that can

be used throughout pregnancy, at relatively low costs. As many of the chromosomal syndromes have similar sonographic findings, a definitive diagnosis cannot be determined based on ultrasound alone, usually requiring follow-up genetic investigations.

Prenatal diagnosis of chromosome abnormalities through the analysis of amniocytes or chorionic villus samples is the standard of prenatal care. The application of chromosomal microarray analysis in routine chromosomal analysis has rapidly and substantially increased the diagnostic yield in clinical cytogenetics.

Materials and Methods: We selected three prenatal cases with minor ultrasound findings and major structural chromosomal abnormalities. Molecular karyotyping analysis was performed with either oligo or SNP-based microarrays developed for the detection of copy-number alterations: microdeletions, microduplications, aneuploidy and unbalanced translocations.

Results: Chromosomal microarray analysis revealed abnormal results for each of the three mentioned cases that are summarized in the table.

Case	Sonographic findings	Genomic microarray result
1	Unilateral multicystic dysplastic kidney	arr[GRCh37]17q12 (34817422_36168104)x1
2	Unilateral club foot	arr[GRCh37]17p11.2 (16657318_20433723)x3
3	Ventriculomegaly	arr[GRCh37]1q42.2q44 (233141951_249205158) x3,13q33.2q34 (106642740_115107733)x1

Conclusions: Identification of any fetal malformation should alert the sonographer for further investigation. Ultrasonographic markers may hint to genetic imbalances associated with severe consequences after birth. Our cases highlight the need of further invasive tests and genetic analysis, as that many rare microdeletion/microduplication syndromes can be present even with a normal sonographic exam. Therefore, the invasive testing remains the gold standard for prenatal diagnosis of chromosomal syndromes.

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E-P01.26
MicroRNA-200 regulated trophoblast invasion by targeting EG-VEGF in preeclampsia

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Preeclampsia is a severe gestational complication characterized by new onset of high blood pressure and proteinuria after 20 weeks of gestation. It is one of the leading hypertensive disorders in pregnant women, affecting 2 to 8% of pregnancies worldwide. Recently, endocrine gland-derived vascular endothelial growth factor (EG-VEGF) was regarded as a critical factor for embryo implantation and placental development. Micro-RNA 200 family, including miR-200a, -200b, -200c, -141- and -429, were highly expressed in human placenta and maternal circulation during pregnancy. Both EG-VEGF and miR-200 family have been shown to be associated with several pregnancy complications, including abnormal embryo implantation, intrauterine fetal growth restriction, preterm birth and pre-eclampsia. Besides, miR-200 family was predicted to target on 5'UTR of EG-VEGF. In order to investigate the roles of miR-200 family and EG-VEGF in preeclampsia, we analyzed these miRNAs and EG-VEGF expression in the pregnant women of healthy control (n=55) and pre-eclampsia (n=33). The expression level of miR-141 and miR-200a in preeclamptic women was significantly higher in maternal blood ($p < 0.05$), while EG-VEGF was significant lower in the maternal blood and placenta of pre-eclamptic women ($p < 0.05$). Further, we verified miR-141 and -200a were target on 5'UTR region of EG-VEGF and affect the migration and invasion ability of trophoblast (HTR-8/SVneo). The results suggest miR-141 and -200a target on EG-VEGF and inhibit trophoblast migration and invasion, and may therefore predispose to develop pre-eclampsia in human early pregnancy.

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E-P01.27
Association of oxidative stress-related genes with miscarriage

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Introduction: 15% of all human pregnancies end in miscarriage before 12 weeks of gestation. Oxidative stress may play a very important role in the miscarriage with unknown etiology. This study was conducted to investigate the association of polymorphisms in oxidative stress-related genes with miscarriage.

Materials and Methods: A total of 127 women with miscarriage and 138 controls were genotyped for SOD1 rs4998557, SOD2 rs4880, CAT rs1001179, GPX4 (rs713041), EDN1 rs5370 and NOS3 rs2070744.

Results: A protective effect of SOD1 rs4998557-G allele on spontaneous abortion was shown in individual SNP

analysis: P=0.03, OR = 0.49, 95% CI 0.26-0.93. It was established that the genotype AsnAsn EDN1 rs5370 was associated with an increased risk of missed abortion in the first trimester (OR 6.7, 95% CI 1.3-34.2). The multi-factor dimensionality reduction approach revealed gene-gene interactions for NOS3, GPX4 and EDN1 genes on spontaneous abortion. Cumulative gene risk score analysis demonstrated that genotype LysLys198 EDN1 /-786TT NOS3 / 718CC GPX4 was associated with spontaneous abortion (P=0.003, OR = 4.28, 95% CI 1.63-11.2). The missed abortion was associated with interaction of GPX4, NOS3 and SOD2 genes. Cumulative gene risk score analysis demonstrated that more than three risk alleles in the genes GPX4 (rs713041-T), NOS3 (rs2070744-C), SOD2 (rs4880-Val) were associated with missed abortion (P = 0.038, OR = 4.23, 95% CI 1.2-15.1).

Conclusion: Gene-gene interactions of oxidative stress related genes are able of modulating of miscarriage risk. This study was supported by the federal assignment № 6.6762.2017 from Russian Ministry of Science and Education.

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The incidence of particular types of chromosomal numerical aberrations in miscarriages is associated with maternal age

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It is commonly known that the risk of giving birth to a child with trisomy 13, 18 and 21 increases rapidly with the maternal age. The relationship between advanced maternal age and incidence of Down syndrome was initially reported more than 75 years ago. On the other hand it has been proven that maternal age has no impact on the incidence of monosomy X in liveborns.

In this study 579 unselected products of conceptions were analysed using commercially available kits for chromosomes 13, 15, 16, 18, 21, 22, X and Y (QF-PCR) and for all chromosomes (MLPA)In contrast to QF-PCR, MLPA is unable to detect 69,XXX triploidy. Overall 170 different trisomies, 38 monosomies X, 40 triploidies and 8 double aberrations were detected using MLPA or QF-PCR. Using MLPA, trisomies of all chromosomes except of chromosome 1 and 19 were diagnosed and the most common trisomies were of chromosome 16, while for QF-PCR the most common aberration was monosomy X. Similarly as in previously described studies, average age of women with trisomic pregnancy was significantly higher than in women with euploid pregnancy (35.73 vs 33.63 p<0.005) and the difference in maternal age for euploid foetuses and foetuses with X monosomy was insignificant (32.73 vs 33.63, p = 0.31). Interestingly, frequency of triploidy in aborted foetuses seemed to decrease with maternal age (average age 31.23 vs 33.63, p<0.05). Meiotic error in egg is a leading genetic cause of trisomy, however still little is known about the molecular basis of aneuploidies related to maternal age.

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E-P01.31

Results of prospective study of utilisation of Trisomy test in noninvasive prenatal testing for trisomies of chromosomes 21, 18 and 13

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Introduction: Noninvasive prenatal testing of most common trisomies, based on analysis of circulating DNA from blood of pregnant women, becomes an important part of prenatal screening.

Aim: Aim of the work was prospective study of utilisation of Trisomy test for noninvasive prenatal testing (NIPT) of the most common trisomies of chromosomes 21, 18 and 13.

Materials and Methods: From September 2015 till April 2017, 4109 samples of pregnant women were analyzed using Trisomy test. For high risk samples detection whole genome low coverage scan was used in association with home-made bioinformatic pipeline and our own biostatistical approach.

Results: Of 4109 analysed samples 3847 were reported as euploid and 76 as trisomic. After analysis of the first blood sample, 184 cases were found to be nonreportable, after second blood sample analysis only 42 samples were still unreportable, so no call rate of the test was 1%. Among

trisomic samples 55 samples were reported as high risk for trisomy 21, 15 samples as high risk for trisomy 18 and 6 samples as high risk for trisomy 13. Two false negatives (1x T21 and 1x T18) and two false positives (1x T21 and 1x T13) were recorded in the whole cohort. Total sensitivity of the method used for detection of all three trisomes was 97.37%. Total specificity of the method was 99.95%.

Conclusions: Trisomy test performance based on calculations of its sensitivity, specificity and no call rate is fully comparable with other commercial tests used in NIPT worldwide.

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E-P01.32
NLRP7 affects the differentiation of human decidual macrophages

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Introduction: The decidual M2 macrophage plays a vital role in the establishment and maintenance of pregnancy. It is different from the abundant M1 macrophages existing in deciduae of spontaneous abortions. However, it is unknown what the molecular regulator of decidual macrophage differentiation is. In our published studies: MPA (Medroxyprogesterone acetate; a progesterone analog with an anti-inflammatory property) drove macrophages differentiating toward a phenotype of decidual M2 macrophage; also, NLRP7 expressed in the decidualized endometrial stromal cells induced by MPA. NLRP7 has a well-studied role in regulating immune responses. Therefore, this study aimed to examine whether NLRP7 plays a role in decidual macrophage differentiation.

Materials and Methods: We detected the expressions of M1-cell markers (IL-1β, TNF-α, and iNOS) and M2-cell markers (IL-10, IDO, and MRC) respectively in LPS/IFN-γ and IL-4/IL-13 inducing the PMA primed THP-1 cell line, which was either siNLRP7 knockdown expressed or LV-NLRP7 overexpressed. We also examined the subtypes of the decidual macrophages by immunofluorescence staining in paraffin embedded the first-trimester endometrium.

Results: The expressions of M1-cell markers are significantly lower in LV-NLRP7 overexpressed and higher in siNLRP7 knockdown expressed THP-1 cell line. The expressions of M2-cell markers slightly increase and decrease in LV-NLRP7 overexpressed and siNLRP7 knockdown expressed THP-1 cell line, respectively. NLRP7 expresses in IL-$10^{+}$$CD68^{+}$ M2 macrophage of the first-trimester endometrium.

Conclusions: We suggest that NLRP7 suppressed the differentiation of M1 macrophages in deciduae. However, NLRP7 was necessary but not sufficient for decidual M2 macrophages differentiation. This research was supported from MOST of Taiwan (103-2314-B-006-079-MY3).

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E-P01.33
Increase of selected single chromosome aneuploidies in embryos of older mothers

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Preimplantation genetic testing for aneuploidy (PGT-A) on day-5 embryos by VeriSeq PGS Kit is routinely used in Gennet since 2016. In 2017 trophectoderm biopsies of 2767 embryos in 939 IVF cycles were performed with 98% examination success rate; 2631 examined embryos. Overall 1295 euploid embryos (49%) were recommended for the

embryotransfer and 190 mosaic embryos (7%) were consulted with clinical geneticist. Five age groups were established for the evaluation of the maternal age-dependent aneuploidy frequency: I.<29 (mean age 26.8), II. 30-34 (32.6), III. 35-39 (37.4), IV. 40-44 (41.6) and V.>45 (46.8). The embryos of the oocyte donors were used as a control group VI. (24.9). Unsurprisingly, the aneuploidy frequency in the age groups III, IV and V compared to the control group VI was significantly higher (t-test, α level 5%), with significant growth of the incidence of the aneuploid embryos from group I to V (P=0.05). The relevant age-dependent increase of single chromosome aneuploidy per embryo was found in monosomies 15, 16, 21, 22 and trisomies 16, 19, 21, 22. The linear correlation was found only for chromosome 15 monosomy (P<0.05). The groups I to III had the exponential increase of single chromosome aneuploidy for chromosomes 16, 19 and 22. When compared to the frequency of the aneuploidies in the spontaneous abortion, the frequent 16, 19 and 22 trisomies and 15, 16 and 22 monosomies are likely lethal for the embryos. Our results provide important clues for the assessment of the potential risk for the embryotransfer of the mosaic embryos.

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E-P01.34
Preimplantation genetic testing (PGT-M) in the family with *SMN1* duplication/deletion

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Spinal muscular atrophy (SMA) is characterized by muscle weakness and atrophy resulting from progressive degeneration and loss of motor neurons in the spinal cord and the brain stem. SMA is caused in more than 95% cases by recessive mutation in the SMN1 gene resulting in deficiency of SMN (survival motor neuron) protein. Approximately 95-98 % of SMA patients lack both copies of *SMN1* exon 7. About 3% of carriers have *SMN1* duplication *in cis* associated with *SMN1* deletion on homologous chromosome. *SMN1* duplication/deletion *in cis* cannot be detected by MLPA commonly used at carrier screening.

We present the case of *SMN1* exon 7 del carrier couple in which risk haplotype suitable for PGT-M may not be unambiguously determined by direct MLPA *SMN1* testing. Male partner has typical heterozygous *SMN1* exon 7del genotype as well as his parents. His affected sister is homozygous. Female partner and her brother are heterozygotes of *SMN1* exon 7del, but their both parents have two *SMN1* copies.

We assumed that one of parents of female partner is false-negative carrier – has SMN1 duplication/deletion. By combination results of MLPA analysis and comparing haplotypes of all relatives of female´s family we conclusion that mother of female partner is heterozygous for *SMN1* deletion with SMN1 duplication *in cis*.

The linkage analysis is available for families with not sufficiently informative results of direct testing. This method may be used for the confirmation of results from carrier screening or for PGT-M preparation as mentioned above.

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E-P01.36
Decoding the role of regulatory element polymorphisms in preeclampsia

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Preeclampsia is a common pregnancy-specific disorder with unknown etiology. It is the leading cause of maternal and perinatal morbidity and mortality. We used a new approach to detecting genetic markers of preeclampsia based on a combination of genomic, transcriptomic, and bioinformatic methods. Our prior genome-wide transcriptional profiling of placental tissue led to a novel set of 63 preeclampsia candidate genes (differentially expressed genes, (Fold Change >1.5, FDR<0.1)). In this report, we present study on the role of variability in these genes in the genetic susceptibility to preeclampsia. We analyzed 48 regulatory single nucleotide polymorphisms (rSNPs) in 23 genes (*ANKRD37, BCL6, BHLHE40, CEBPA, CCSAP, CORO2A, ENG, GPT2, GSTA3, HK2, INHA, KRT19, LEP, LHB, NDRG1, PLIN2, PPP1R12C, RDH13, SASH1, SIGLEC6, SYDE1, TMEM136* and *ZNF175*) in 519 patients with preeclampsia and 718 women with uncomplicated pregnancies from Russian, Buryat and Yakut populations using MassArray iPLEX (Sequenom). We have detected significant associations for preeclampsia with eleven rSNPs in *PLIN2, BHLHE40, RDH13, SYDE1* and *ZNF175* genes in Russian

and Buryat population. In Yakut population, only three genes *(NDRG1, CORO2A, SASH1)* were associated with increased risk of preeclampsia. rSNP in *PPP1R12C* genes was associated with preeclampsia in Buryat population only. This results demonstrate a significant role rSNP of placental tissue genes in susceptibility to preeclampsia in different ethnic groups of Russia. Integrative transcriptome-based approach proved its efficiency and may be applied to detecting new potential genetic markers of preeclampsia, which reduces missing heritability. This work was supported by the Russian Foundation for Basic Research (grant №18-44-700007).

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Analysis of genes associated with preeclampsia for purposes of the disease prognosis

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Introduction: Preeclampsia is a serious and poorly understood complication of pregnancy and remains one of the leading cause of maternal and perinatal mortality and morbidity. It is a multi-systemic disorder usually recognised by new-onset of hypertension and proteinuria in the second half of pregnancy. Although the etiology of preeclampsia is not elucidated yet, the placenta plays an important role in the pathogenesis. There are many genes and biochemical markers closely associated with placental function which is associated with preeclampsia.

Materials and Methods: Low coverage whole genome sequencing data obtained in NIPT test from pregnant women was mined for identification of candidate genes mostly associated with preeclampsia. Maternal polymorphisms were identified and verified by Sanger sequencing.

Results: We were able to determine allelic frequencies of preeclampsia associated SNVs specifically in our population. In addition, we verified selected polymorphisms associated with preeclampsia by conventional Sanger sequencing after suggesting specific average values.

Conclusion: Population study confirmed our assumptions that using next generation sequencing and Sanger sequencing may, at least partially, extend the possibilities of diagnostics in the field of preeclampsia.

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preimplantation genetic testing for carriers of balanced chromosomal rearrangements

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Introduction: Structural chromosome abnormalities may be associated with infertility, multiple miscarriage and delivery of chromosomally unbalanced offspring. Preimplantation genetic testing for structural rearrangements (PGT-SR) was used to select embryos without chromosomal imbalance for transfer.

Materials and Methods: We conducted 13 cycles of IVF +PGT-SR. A-CGH (Illumina 24Sure+) was used to detect structural rearrangements of chromosomes and whole chromosome aneuploidies.

Results: PGT-SR was performed for 48 blastocysts. In 6 cycles for robertsonian translocation carriers 28 blastocysts were obtained, 13 from them had normal/balanced chromosome number (46,4%). After 4 embryo transfers 3 patients achieved pregnancy. The first pregnancy resulted in delivery of a healthy child and 2 pregnancies are prolonging. In 5 cycles for reciprocal translocation carriers 14 blastocysts were obtained, 2 from them (from different patients) had normal/balanced chromosome number (14,3%). After 2 embryo transfers both patients became pregnant. In 2 cycles for inversion carriers we obtained 6 blastocysts, in both cases there was one blastocyst with normal/balanced chromosome number (33,3%). One patient has ongoing pregnancy, the other one is waiting for embryo transfer.

Conclusions: Transfer of embryos without chromosomal imbalances is important factor for successful implantation, pregnancy and delivery a healthy baby. IVF+ PGT-SR is effective method for carriers of balanced chromosomal rearrangements that can be used for the earliest prevention of hereditary pathology.

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E-P01.40
Results of karyotype analysis of 7975 pregnancies prenataly identified with amniocentesis in Turkey

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Introduction: 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.

Materials and Methods: A retrospective review of our amniocentesis database for the period from January 2000 to February 2018 was carried out. The karyotyping of 7975 fetuses was carried out in Department of Medical Biology from the samples of amniotic fluids which were sent from Department of Gynecology and Obstetrics of Balcali Hospital. A standart nomenclature has been developed to describe each of types of abnormality found in human chromosomes.

Results: A total of 7975 amniocentesis specimens were processed during the study period. 545 fetuses (6.83%) had various chromosomal abnormalities. 56.33% of abnormal karyotypes (307 cases) were numerical and 41.46% (226 cases) were structural. Both numerical and structural chromosomal aberrations were observed in 12 cases (2.20%). The ratios were as: trisomy 21 (46.90%), trisomy 18 (18.24%), monosomy X (10.42%), trisomy 13 (6.84%), Triploidy (5.21%), Klinefelter Syndrome (3.58%), Trisomy X (1.30%), XYY Syndrome (0.97%), and the others in all numerical abnormalities. The frequent structural abnormalities were as: 46,XX/XY,inv(9)(p11;q12)/(p11;q13) (33.18%), 46,XX/XY,1qh(+)(10.17%), 46,XY,Yqh(-) (5.30%), 46,XX/XY,16qh(+)(4.86%), 46,XX/XY,9qh(+) (4.42%) and 46,XY,Yqh(+) (4.98%). 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 6.83%. This ratio emphasize the importance of prenatal diagnosis.

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Prenatal diagnosis of foetuses with cardiac sonographic abnormalities by array comparative genomic hybridization

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Congenital heart defects occur in 8-10 out of 1000 live born newborns and are one of the most common causes of their death. In fetuses, congenital heart defects are found 3-5 times more often. Currently, Array Comparative Genomic Hybridization (array CGH) is recommended by the American College of Obstetricians and Gynecologists as a first line test in the prenatal diagnosis of fetuses with sonographic abnormalities especially cardiac defects.

We present the results of oligonucleotide array application in a cohort of 353 prenatal cases with the fetal cardiac abnormalities, detected by ultrasound scan. In all cases amniocentesis was performed at 12-24 weeks of gestation. DNA was extracted from amniotic fluid and trophoblast. Array CGH was performed using 60K microarrays 8x60K from Oxford Gene Technology (CytoSure ISCA, v2 and v3).

The most commonly found (56%) are aneuploidy: chromosomal trisomy 21, 18, 13 and Turner syndrome. In cases with aneuploidy, the most common heart malformations are atrioventricular septal defect (AVSD), ventricular septal defect (VSD), tetralogy of Fallot (TOF), atrial septal defect (ASD) and hypoplastic left heart syndrome, (HLHS). Heart defects are a characteristic phenotypic feature in common microdeletion/microduplication syndromes (34%), for example in the cases with deletion syndrome 22q11.2 the most commonly defects are TOF, VSD and aortic coarctation (CAT). Rare aberrations accounted for 10% of all abnormal results.

Prenatal array-CGH is the only method permitting to the identification of all unbalanced aberrations (number and structure) with a much higher resolution than the currently applied traditional assessment techniques karyotype.

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Detection of chromosomal anomalies from South of Turkey: QF PCR versus cytogenetic

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Introduction: In the early 1990 s, FISH and, more recently, QF-PCR entered to the field of prenatal diagnosis, to overcome the need of fetal cell culturing, hence to allow rapid diagnosis of some selected chromosomal anomalies. Rapid QF-PCR analysis for the common trisomies 13, 18, and 21 and sex chromosome aneuploidy is now offered in many centers to women, who undergo invasive prenatal diagnosis by amniocentesis or chorionic villus sampling. This is usually performed in addition to the subsequent full chromosome analysis of cultured cells.

Materials and Methods: The karyotyping and QF-PCR were carried out to amniotic fluids of 1380 fetuses from January 2013 to January 2018. The diagnosis of common chromosomal numerical and structural anomalies were routinely performed by standard cytogenetic techniques. The QF-PCR amplifications were performed using trisomy detection kit. The results from both methods were compared in terms of structural anomalies.

Results: In our retrospective study, we detected 135 cases with numerical (48/135) and structural (87/135) anomalies. The various types of structural anomalies were found with conventional cytogenetic methods in 87 cases, who, however, were found normal with QF-PCR. No false aneuploidic results were observed by QF-PCR method and two methods are in accordance with each other. **Discussion:** Aneuploidic QF-PCR results were validated with cytogenetic analyses to complete the diagnosis. The QF-PCR is an easier and a faster test, it has a limitation of not to be able to scan full karyotype. In conclusion, QF-PCR and cytogenetic methods are important prenatal tests that completes each other.

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Distribution of *FMR1* and *FMR2* repeats in Argentinean patients with primary ovarian insufficiency

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Introduction: the premutation state of *FMR1* (Fragile X Mental Retardation 1) has been associated with primary ovarian insufficiency (POI), and is the most common known genetic cause for 46,XX patients. Nevertheless, very few studies have analyzed its frequency in Latin American populations.

Additionally, a relationship between alleles carrying a cryptic microdeletion in the 5'UTR of *FMR2* and the onset of POI has only been studied in one population. Our aim was to analyze the incidence of *FMR1* premutations and putative microdeletions in exon 1 of *FMR2* in a cohort of Argentinean women with POI.

Materials and Methods: we studied 133 patients and 84 controls. Fluorescent PCR was performed, and the *FMR2* exon 1 was further sequenced in samples presenting less than 11 repeats.

Results: we found the frequency of *FMR1* premutations to be 6.7% and 2.9% for familial and sporadic patients, respectively. Among controls, 1/84 women presented a premutation. In addition, although we did not find microdeletions in *FMR2*, we observed a change (T >C) adjacent to the repeats in two sisters with POI. Given the repetitive nature of the sequence involved, we could not ascertain whether this represents a single nucleotide polymorphism (SNP) or a deletion.

Conclusion: a relationship between *FMR2* and POI could not be established for our population.

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Association analysis of *HLA-G* (rs41557518) and *VEGF-A* (rs2010963) polymorphisms with recurrent miscarriages

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Recurrent miscarriage (RM) is defined as the loss of 2 or more clinical pregnancies and affects 15-20% of couples. Nearly 50% RM cases remain idiopathic. A successful pregnancy requires coordinated interaction of several components between mother and fetus. It has been postulated that abnormal maternal and fetal expression of genes which are associated with immunity, angiogenesis and apoptosis may be contributing to cases of recurrent miscarriages. Vascular Endothelial Growth Factor-A (VEGF-A) protein is an antiapoptotic, mitogenic and major angiogenic factor and a prime regulator of endothelial cell proliferation. It plays an essential role in physiological vasculogenesis and vascular permeability. Human leukocyte antigen *(HLA)-G* gene is a non-classical HLA class Ib gene and favours maternal acceptance of the semi-allogenic fetus by modulating the maternal immune system during pregnancy. The present study determines the association of *HLA-G* (rs41557518) and *VEGF-A* (rs2010963) polymorphisms in 100 RM women who had experienced at least two or more consecutive miscarriages, their 100 male partners and 100 healthy control females who had at least one healthy live born and no miscarriage. The genotyping was performed by using AGENA MassARRAY. The variants showed distribution following Hardy-Weinberg equilibrium ($p>0.05$). The variant rs41557518 showed a significant association ($p<0.05$) for recurrent miscarriages when compared between RM women and control women. Further investigations with increased sample size may provide conclusive results about the association of these variants with recurrent miscarriages. The financial assistance to Vishali Kalotra (DST/ INSPIRE Fellowship/ 2013/ 889) from Govt. of India is duly acknowledged.

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E-P01.46

Increased risk for recurrent pregnancy loss in FXIII V34L and PAI-1 4G/5G compound heterozigots

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Recurrent pregnancy loss (RPL) is a heterogeneous condition affecting up to 5% of women of reproductive age. Proper formation of placenta is necessary for successful pregnancy outcome. It has been postulated that FXIII V34L and PAI-1 4G/5G polymorphisms can interfere with fibrinolysis and fibrin cross-linking, and thus contribute to pregnancy loss. Here we examined the prevalence of aforementioned polymorphisims among women with history of recurrent miscarriages and fertile controls.

The study included 70 women with history of at least two early pregnancy losses (before 20th gestation week) and 30 fertile controls with no miscarriages. We investigated polymorphisim in FXIII (V34L) and PAI -1 (4G/5G) genes using reverse PCR Vienna lab CVD StrippAssays.

Our results showed no statistically significant difference in prevalence of FXIII and PAI-1 gene polymorphisms between two tested groups. However, relative risk for PRL among women heterozygous for FXIII V34L was 2.81 times increased (CI 95%, p 0.02). Using stepwise logistic regression analysis we showed that combined presence of high risk genotypes for FXIII and PAI-1 increases relative risk for RPL 13.98 times (CI 95%, p 0.04).

FXIII V34L polymorphism leads to change in fibrin structure making it more resistant to fibrinolysis. In combination with decreased fybrinolitic activity of PAI-1 4G variant it can lead to improper formation of placenta. Therefore, compound heterozygots for FXII V34L and PAI-1 4G can have increased risk for RPL.

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Single nucleotide polymorphisms of vitamin D receptor and recurrent miscarriage

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Introduction: Recurrent miscarriage (RM) is a reproductive disorder defined as the loss of two or more pregnancies from the time of conception until 22 weeks of gestation. Despite the fact that several mechanisms have been previously described for the pathogenesis of RM, causes of approximately 50% remain unknown. Recent studies indicate association of vitamin D with adverse pregnancy outcome, including RM. The vitamin D receptor (VDR) is a crucial mediator of the pleiotropic cellular effects of vitamin D and its function is influenced by several single nucleotide polymorphisms (SNPs). The main objective of the present

study was to assess whether three different VDR SNPs are associated with the risk of RM in Slovenian women.

Materials and Methods: A case - control study was designed to examine the potential association of VDR polymorphisms (FokI rs222857, Cdx2 rs115688, Taq1 rs731236) with RM. A total of 149 women with a history of ≥3 spontaneous miscarriages of unknown aetiology were included in the study. The control group consisted of 149 age-matched, healthy women with at least two live births. Genotyping was performed using PCR-RFLP methods.

Results: No statistically significant differences were found in the distribution of genotype and allele frequencies of either SNP between patients and controls or patients with primary and secondary RM. Moreover, we found no association between abovementioned SNPs and RM under dominant, recessive and codominant genetic models.

Conclusions: Our results suggest that VDR gene polymorphisms are not a genetic marker for RM in Slovenian women. **Research grant numbers:** 16.06.2.1.02, 13.06.1.3.32

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Prenatal diagnosis of ring chromosome 4 and Wolf-Hirschorn syndrome with bilateral polycystic kidney disease

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Ring chromosomes are unusual abnormalities that are observed in prenatal diagnosis. A 34-year-old patient referred for amniocentesis due to abnormal maternal serum screening result in the 16th week of pregnancy. Cytogenetic analysis of cultured amniyotic fluid cells revealed out ring chromosome 4. Both maternal and paternal karyotypes were normal. Terminal deletion was observed in both 4p and 4q arms of ring chromosome 4 by fluorescence in situ hybridization (FISH). Also deletion was observed in the Wolf-Hirschorn Syndrome (WHS) critical region of ring chromosome 4 by an additional FISH study. These results were confirmed by means of microarray analysis showing terminal deletions on 4p16.3 (8412 Mb) and 4q35.2 (5361 Mb). A girl infant born at 36-week gestational age through ceaserean section with severe intrauterine growth retardation, dysmorphic facial features, hypoplasia of the corpus callosum and bilateral polycystic kidney disease diagnosed within prenatal period. Our report describes the prenatal case with a ring chromosome 4 abnormality with Wolf Hirschorn Syndrome completely characterized by array-CGH which provided complementary data for prenatal diagnosis and genetic counseling of this syndrome.

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Co-occurrence of two rare disorders in a non-consanguineous family

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Introduction: Spinal muscular atrophy (SMA) and Alport syndrome are rare genetic conditions characterized with different symptoms and outcomes. Here we present a rare case with co-occurrence of these two unrelated disorders in one family. The family came to our attention because of SMA diagnosed in their first child. A heterozygous variant *COL4A5*:c.4228C>T (p.Arg1410Cys), associated with Alport syndrome, was found accidentally in the mother through NGS. The family has several further pregnancies with different outcomes: two miscarriages, one healthy girl born after prenatal diagnosis and one terminated pregnancy with SMA.

Materials and Methods: The CVS sample from the sixth present pregnancy with a male fetus was first examined for deletion of exons 7 and 8 of the *SMN1* gene by RFLP analysis. Exon 46 of *COL4A5* was investigated then in the fetus and the mother's father by targeted amplicon sequencing on an Illumina MiSeq system.

Results: A homozygous deletion of exons 7 and 8 of the *SMN1* gene was not detected. The grandfather and male fetus were found to be hemizygous carriers of the variant *COL4A5*:c.4228C>T.

Conclusion: The X-linked recessive form of Alport syndrome is progressive but characterized with large clinical heterogeneity and variable age of onset in affected males. Despite unilateral hearing loss, arterial hypertonia

and visual problems, a diagnosis of Alport syndrome is not established in the grandfather. Difficult ethical questions concerning the decision about this pregnancy outcome and the health of the unborn child are raised in this rare case with co-occurrence of two rare disorders.

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E-P01.50

Can tangier disease cause male infertility? A case report and an overview on genetic causes of male infertility & hormonal axis involved

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Introduction: Tangier disease is an autosomal recessive disorder caused by mutations in the *ABCA1* gene and characterized by the accumulation of cholesteryl ester in various tissues and a near absence of high-density lipoprotein. The subject in this investigation was a 36-year-old man with Tangier disease and with a severe oligoasthenoteratozoospermia diagnosis. Testosterone is the sex hormone necessary for spermatogenesis and cholesterol is its precursor; hence, we hypothesized that the characteristic cholesterol deficiency in Tangier disease patients could compromise their fertility. The aim of the study was to therefore to determine if there is an association between Tangier disease and male infertility.

Materials and Methods: After excluding viral, infectious, genetic and anatomical causes of the subject's oligoasthenoteratozoospermia, we performed a hormonal analysis to verify our hypothesis.

Results: The patient was found to be negative for frequent bacteria and viruses. The subject showed a normal male karyotype and tested negative for Yq microdeletions and Cystic Fibrosis Transmembrane Conductance Regulator gene mutations. A complete urological examination was performed, and primary hypogonadism was also excluded. Conversely, hormonal analyses showed that the subject had a high level of follicle stimulating hormone and luteinizing hormone, low total testosterone and a significant decline in inhibin B.

Conclusion: We believe that the abnormally low cholesterol levels typically found in subjects with Tangier disease may result in a reduced testosterone production which in turn could affect the hormonal axis responsible for spermatogenesis leading to a defective maturation of spermatozoa.

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E-P01.51

A supernumerary ring chromosome 18 in a fetus with tetralogy of Fallot

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In prenatal diagnosis, small Supernumerary Marker Chromosomes (sSMC) occur in 0.072%. We report here a fetus with a sSMC, identified as a ring chromosome 18, associated with a cardiac defect.

Patient: We present a 36-year old pregnant patient who was referred to the Center for Prenatal Diagnosis at Montpellier University Hospital for a ventricular septal defect, conotruncal type, consistent with a diagnosis of tetralogy of Fallot, revealed on 32 weeks' gestation ultrasound screening.

Results: Rapid interphase screening performed on 133 uncultured amniocytes showed 58 nuclei with 3 signals for centromere 18 probe (D18Z1). Surprisingly the karyotype showed a mosaic ring chromosome 18, dicentric in some metaphases, objectived by additional FISH analysis with D18Z1 probeConsequently, the chromosomal formula was established as follows:47,XX,+r(18)(p11.22q11.2)[19/20]/46,XX[1/20].ish r(18)(wcp18+,D18Z1+)[11/49]/r(18)(wcp18+,D18Z1++)[3/49]Array-CGH analysis was performed to precise chromosomal content. It revealed the presence of a 5.9-Mb duplication at chromosomal region 18p11.22p11.21 and a second one of 5.8-Mb at chromosomal region 18q11.1q11.2 encompassing the entire gene, *GATA6*. Mutations in *GATA6* are described in cases of tetralogy of Fallot and some authors argue that *GATA6* gain can be observed in patients with conotruncal defect, suggesting a phenotype-gene dosage relationship.

Conclusion: To date, only five cases of supernumerary ring chromosome 18 have been reported with a wide phenotypic range due to variations in genetic content. In our case, we identified *GATA6* as a good candidate gene to explain cardiac defect. We suggest that molecular characterization of sSMC may help genetic counseling and a better understanding of chromosomal imbalances.

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E-P01.53

Family case of Turner syndrome in ring X chromosome mosaicism

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Clinical case: We report a family (mother, 52 years and two her daughters - 32 and 25 years) with TS. The patients had a similar phenotype: short stature (mother's height - 140 cm, weight - 52 kg; daughter's height - 138/140 cm, weight - 38/39 kg), narrow palate, a low-set and posteriorly rotated ears, short neck, low posterior hair line, shield shaped chest, scoliosis, normal intelligence. The mother had menarche at 13 y.o., and menopause at 45 y.o., 5 pregnancies ended 2 miscarriages, 1 medical abortion and two 2 births (2 daughters). They had a regular menstrual cycle. Older daughter was infertile because of obstruction of the fallopian tubes, younger daughter was unmarried.

Materials and Methods: Chromosome analysis was done on peripheral blood lymphocytes. FISH analysis with the DNA probes (DXZ1, KAL Xp22.3) was performed on peripheral blood lymphocytes and buccal smear cells. At least 100 cells were analyzed for each FISH analysis.

Results: Mosaic ring (X)(p22.3q28).ish (DXZ1+,KAL +) was detected in mother and her daughters. The patients presented 45,X/46,Xr(X) mosaicism with similar proportions of cells containing the r(X) in the lymphocytes and buccal smear cells: 8% and 26% (mother), 9% and 40% and 11% and 47% (daughters, 32 and 25 y.o.), respectively. The proportion of the cell lines with and with no ring X chromosome is between tissues in the same TS patient. There is a positive correlation between age and percentage (%) of r(X) in epithelia cells. There is no similar correlation for mosaicism in the lymphocytes.

N.V. Oparina: None. **O. Pilyaeva:** None. **S. Kalinenkova:** None. **A. Latypov:** None. **V. Chernykh:** None.

E-P01.55

Which embryo should be transferred after preimplantation genetic diagnosis (PGD) for X-3 reciprocal translocation in male patient?

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Introduction: Balanced autosome-gonosome translocations occur in about 1/30,000 live births. In women with a balanced X-autosome translocation, the normal X chromosome is preferentially lyonised and the phenotype is normal but gene silencing may occur on X-autosome translocated chromosome. The phenomenon is unpredictable and has as consequence from premature ovarian failure to major genetic disorders and mental retardation.

Materials and Methods: The female patient had normal karyotype. The male patient had a karyotype with balanced translocation 46,Y,t(X;3)(p11.2;p14)mat, and a normal phenotype. The female patient underwent two cycles of ovarian stimulation. ICSI was performed on vitrified/warmed and fresh oocytes. Embryos were biopsied at blastocyst stage, on day 5. Chromosomal analysis was performed by Next Generation Sequencing.

Results: Eleven blastocysts were obtained and biopsied from 23 vitrified/warmed and fresh metaphase II oocytes. Two embryos were diagnosed 46,XY, two embryos were diagnosed 46,XX; four embryos were diagnosed 3p14→cen→3qter and monosomy Xp11.2→cen→Xqter, from 2:2 segregation and adjacent-2 disjunction. Three embryos were diagnosed aneuploid [45,X0;45,XY,del(2);46,XY,del(8)(qter →q22.1)]. We knew that the two 46,XX embryos inherited the balanced translocation from the father and the two 46,XY embryos had a normal karyotype. Because the phenotype of balanced translocated female embryos could not be predicted, the couple asked to have a 46,XY embryo transferred. Clinical pregnancy was obtained.

Conclusions: Proposing PGD-SR for gonosome-autosome reciprocal translocation implies the risk to exclude balanced translocated female embryos with a normal phenotype for transfer because the early and late normal development at post-natal stage cannot predicted based on by the only chromosomal analysis.

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E-P01.56

Prevalence of Y microdeletions in Turkish azoospermia or oligospermia patients

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Infertiliy is considered to be a reproductive system disease. It is estimated to affect 15% of couples worldwide. Male factors contributing to infertiliy makes up around 40-50% of the cases. Y chromosome infertiliy makes up 5-10% of the cases with azoospermia or severe oligospermia. Y chromosome infertiliy is caused by the deletions azoospermia factor (AZF) region located in proximal Yq.

Purpose of this study is to determine the frequencies of AZFa, AZFb or AZFc deletions among the males with azoospermia or oligospermia referred to our laboratory for further evaluation between 2009-2018.

Our study consisted of 1926 male patients with azoospermia or oligospermia. We used ChromoQuant® AZF kit to detect the deletions. For AZFa sY86 and sY84, for AZFb sY127 and sY134, for AZFc sY160, sY254 and sY255 markers were utilized.

Among 1926 number of patients, 116 (6.2%) of them had at least one of AZFa, AZFb or AZFc deletions. The most common AZF deletion was AZFc deletion which was detected in 50.8% (n=59) of the patients. The others were AZFb+c in 16.3% (n=19), AZFa+b+c in 15.5% (n=18), AZFa in 11.2% (n=13) and AZFb in 6% (n=7) of patients, respectively.

Percentage of Y microdeletions might be higher in more select and homogeneous populations. It is hopeful that majority of AZF deletions were AZFc microdeletions in our study, since males with AZFc deletions have an oppurtunity for fertility via TESE.

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E-P01.57
Y-chromosome haplogroup architecture confers susceptibility to AZFc microrearrangements

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Introduction: The lack of the entire AZFc region has long been shown to cause azoospermia or severe oligozoospermia, however, it is still arguable to what extent AZFc microrearrangements contribute to impaired spermatogenesis. The aim of our study was to investigate the potential risk AZFc microrearrangements may deliver to spermatogenesis and to ascertain the possible association of Y-haplogroups with distinct AZFc microrearrangements and spermatogenic impairment.

Materials/methods: A total of 486 men were examined, mainly Macedonians and Albanians, among which 338 were azoospermic/oligozoospermic and 148 normozoospermic. The AZFc microrearrangements were analyzed with STS and SFV markers, QF-PCR and MLPA analysis. Additionally, we determined the Y-haplogroups of all subjects using direct SNP typing and indirect prediction with Y-STR markers.

Results: We observed two microdeletions: gr/gr and b2/b3, three microduplications: b2/b4, gr/gr, b2/b3 and one complex rearrangement gr/gr del.+ b2/b4 dupl. Our results did not show a statistical association of impaired spermatogenesis with microrearrangements, however a significant correlation of specific rearrangements with distinct haplogroups was observed. The b2/b4 and gr/gr duplications were statistically associated with HgR1a ($p = 7.27x10^{-13}$ and $p = 0.0026$ respectively), whereas the b2/b3 deletions were detected almost exclusively in HgE ($p = 0.0051$), implying that the Y-chromosome background confers susceptibility to AZFc microrearrangements. Subgroup analysis based on ethnicity revealed a significant difference between the frequency of Macedonian and Albanian HgR1a b2/b4 duplication carriers ($p = 0.0315$).

Conclusion: These results are specific to a population of Macedonians and Albanians, thus due to the perceived "regionality" of Y-chromosome microrearrangemets and haplogroups this study further adds to the body of literature.

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E-P02 Sensory disorders (eye, ear, pain)

E-P02.01
Spectrum of clinical phenotypes associated with *PAX6* missense mutations

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Introduction: Mutations of the *PAX6* gene cause several phenotypes characterized by complex of ocular malformations varying in expressivity and combination. The most part of loss-of-function (LoF) mutations causes aniridia (absence of the iris among other signs) or closely related phenotypes, while the majority of missense mutations could lead to several other conditions. To make steps towards investigation of phenotype-genotype correlations, the clinical picture of 6 patients with different missense mutations in the *PAX6* gene is analyzed.

Materials and Methods: A cohort of patients with *PAX6* missense mutations underwent ophthalmological examination and molecular diagnosis.

Results: Two mutations cause aniridia phenotype, three others result in closely related phenotypes. A single missense is associated with iris damage different from aniridia (see Table). A carrier of the mutation also has retinal glial layer atrophy, macula hypoplasia, abnormal retinal pigment epithelium structure, nystagmus and achromatopsia.

Conclusions: A significant variety of clinical picture might be partially explained by different etiopathogenetic mechanisms related to the location and peculiarities of affected amino-acid residue. Functional consequences of missense mutations are to be tested by at least *in vitro* analysis. This work is partially supported by RFBR grant 17-04-00475.

Phenotypes of PAX6 missense mutations

Mutations	Phenotypes
p.(G51R)	Iris hypoplasia, cataract, optic nerve hypoplasia
p.(G7R)	Partial aniridia, cataract, keratopathy, fovea hypoplasia, nystagmus
p.(Q47R)	Partial aniridia, keratopathy, fovea and optic nerve hypoplasia, nystagmus
p.(K55T)	Complete aniridia, cataract, keratopathy, fovea hypoplasia, nystagmus
p.(G72S)	Iris hypoplasia, anterior synechiae, nystagmus, retinal glial layer atrophy, macula hypoplasia, abnormal retinal pigment epithelium structure, achromatopsia
p.(S119R)	Complete aniridia, nystagmus, cataract, fovea hypoplasia

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E-P02.03

A novel founder mutation in the *ABCA4* gene found in Bukharian Jewish population, causing cone-rod dystrophy

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Introduction: Cone-rod dystrophy is a group of eye disorders that affect the light sensitive cells of the retina with prevalence of 1 in 30,000-40,000 individuals. By mid-adulthood most individuals are legally blind. The *ABCA4* gene encodes an ATP-binding cassette transporter found exclusively in the retina's photoreceptors. Biallelic Mutations in *ABCA4* gene cause cone-rod dystrophy.

Materials and Methods: We have performed exome sequencing in an individual of Jewish Bukharian origin and a family history resembles autosomal recessive with pseudo dominant trait. We performed a segregation study for the disease causing variant and screened this variant in Bukharian Jewish population.

Results: A 20 year old male, currently blind with a clinical diagnosis of cone-rod of consanguineous Jews of Bukharian family was referred for a genetic counseling. Family history includes maternal great-uncle and daughter with a clinical picture similar to the probed. The patient was found to have a novel homozygous mutation c.5059delA in the *ABCA4* gene, leading to a premature stop codon and lack of functioning protein. The parents and a sister were heterozygous carriers for the familial mutation. The c.5059delA mutation was found in 5 individuals out of 169 Bukharian Jewish unrelated individuals, giving an estimated carrier frequency of 1:34.

Conclusions: The prevalence of cone-rod dystrophy due to this founder mutation in Bukharian Jews is expected to be about 1:4600. It is yet unclear whether this specific mutation causes exclusively cone-rod dystrophy or includes the spectrum of retinal disorders caused by other *ABCA4* mutations.

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E-P02.05

A novel SCN9A splicing mutation in a compound heterozygous patient with congenital insensitivity to pain and hyposmia

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Congenital insensitivity to pain (OMIM 243000) is a rare autosomal recessive disorder characterized by the absence of pain perception and hyposmia or anosmia. Different loss of function mutations in SCN9A gene (NM_002977.3), encoding the sodium channel Nav1.7, have been associated with this condition.

A 10-year-old girl with clinical evidences of CIP underwent the genetic analysis of SCN9A. Blood samples were collected from the proband, her unaffected twin and both the parents. Genomic DNA was sequenced by next generation sequencing. Transcript analysis were carried out in order to evaluate the mutation consequence on the mRNA splicing. The cDNA was amplified with a specific primer set and different size fragments separated and isolated on agarose gel, then sequenced by Sanger method.

We selected two mutations in SCN9A: a missense mutation p.Arg896Gln (c.2687G>A), previously described by Cox et al. and a splicing variant c.1108-2A>G, not yet reported in public databases. The proband cDNA sequencing showed the delocalization of the acceptor splice-site 46 bp downstream, within the exon 10, inducing a deletion which results in a premature stop codon. Co-segregation analysis showed that the mother and brother were heterozygous carriers of the missense mutation while the father was heterozygous for the splicing mutation.

The deleterious effect of the missense mutation on the sodium channel activity is caused by the alteration of the pore delimiting structure, which is highly conserved in the evolution. The splicing mutation is a truncating mutation that impairs the expression of Nav1.7 channel functioning, providing the explanation of the phenotype.

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E-P02.06
A novel human mutation associates with increased pain threshold and impaired thermoregulation

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Introduction: Congenital insensitivity to pain is a rare autosomal recessive disease, comprises absence of sensation to noxious stimuli. The aim of this study is to identify and characterize the mutation causing congenital insensitivity to pain in two consanguineous Israeli-Bedouin families.

Materials and Methods: Two patients' medical records were carefully reviewed. Their first-degree cousin parents and siblings underwent a complete physical examination. Genomic DNA was extracted from peripheral blood. Chromosomal microarray analysis, exome capture and sequencing were performed. Data was analyzed for quality, exome coverage, and exome-wide SNP/InDel. Basic bioinformatics analysis was performed using Fabric Genomics to search for variations.

Results: We found a new mutation in the PRDM12 gene that was shared by both patients of the two families. An additional homozygous mutation presented only in one of the patients, in a gene that expresses mainly in the CNS and plays a role in modulating pain and temperature via the opioid system. The mutation leads to deletion of two exons thus resulting in absence of important domains in the protein. The mutation didn't present in 240 control chromosomes in the healthy Bedouin population.

Conclusion: Here we present a new mutation in the PRDM12 gene, thus a novel mutation in a gene that plays an important role in pain regulation. Identifying and characterization of a new mutation in genes that are part of the pain mechanisms can lead to developing a new diagnostic tools, prevention and treatment.

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E-P02.07

Warsaw syndrome: two further cases

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Introduction: Warsaw syndrome is an autosomal recessive cohesinopathy due to biallelic *DDX11* mutations. Until now have been described only seven patients and 7 different mutations. The clinical picture is characterized by: 1) pre and postnatal growth retardation; 2) severe microcephaly; 3) mild to moderate intellectual disability; 4) severe sensorineural hearing loss with cochlear malformations; 5) skin pigmentation anomalies; 6) facial dysmorphisms.

Case report. We report the case of two sisters (5 yo and 3½ yo), born to healthy and non consanguineous parents who came to our Institute for a bilateral sensorineural hearing loss and cochlear hypoplasia. The visit highlighted hyposomia with values lower than the 3rd percentile from the prenatal age and a severe and progressive microcephaly. They showed hyperpigmented straeks and hypopigmented spots enlarging over time, and dysmorphic features: mild bilateral epicanthus, upslanting palpebral fissures, prominent nose with small alae nasi, cup ears, micrognathia, and bilateral clinodactyly. Moreover, they showed a language delay although the IQ tests were for both of them in the normal range. There were no cardiac or abdominal malformations. Since the cell cultures demonstrated an elevated spontaneous and mitomycin C (MMC)-induced chromosomal breakages, and sister chromatid cohesion defects, a DDX11 gene analysis was carried out, showing two novel variants (c.907_920del (p.Lys303Glufs*22) and c.2507T>C (p.Leu836Pro)) predicted to be likely pathogenetic according to the ACMG/AMP 2015 guidelines.

Conclusion. Our case enlarges the knowledge about the clinical and molecular data of Warsaw syndrome. Functional analysis are now in progress to demonstrate the pathogenicity of the mutations.

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E-P02.08

FGF3 gene mutations related to two syndromic Congenital deafness cases: Congenital deafness with inner ear agenesis (Michel aplasia), microtia, and microdontia and Otodental dysplasia

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Introduction: The fibroblast growth factors (FGFs) comprise a family of signaling molecules that regulate cell proliferation, differentiation, and migration during embryogenesis and are essential for appropriate patterning and formation of the developing inner ear. Congenital deafness with inner ear agenesis, microtia, and microdontia is caused by biallelic mutations in the FGF3 gene. Otodental dysplasia is a syndrome of sensorineural deafness and globodontia, which has been associated with heterozygous microdeletions of chromosome 11q13 involving the FGF3 gene.

Materials and Methods: For case one, FGF3 gene sequence analysis using Sanger sequencing method was used whereas array-CGH was performed for case two.

Cases: Case one is a 5 year-old female who was born at term by normal delivery. Her parents were consanguineous. (second cousins). She had a long face, with micrognathia, microtia, microdontia and preauricular skin tags. Case two is a one year-old male who was born at term by normal delivery. He had a long face, long philtrum, anteverted nostrils, cupped shaped ears, preauricular skin tags and single transverse palmar crease on left hand. Percentile values of all anthropometric measurements were between 10 and 25 in both cases and audiologic examination of both cases revealed bilateral sensorineural deafness.

Results: In case one a novel homozygous c.8T>G (p. Leu3Arg) mutation was found in the FGF3 gene. Case two had heterozygous 11q13.3q13.4 microdeletion (2,804 kbp) including FGF3 gene.

Conclusions: Our cases have typical clinic features of FGF3 related syndromic deafness. These cases have been reported to contribute to genotype-phenotype correlations in FGF3 related syndromic deafness.

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E-P02.09

Analysis of *GJB2* mutations and the clinical manifestation in a large Hungarian cohort

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Introduction: Pathogenic variants of the gap junction beta 2 (*GJB2*) gene are responsible for about 50% of hereditary non-syndromic sensorineural hearing loss (NSHL). In this study, we report the mutation frequency of *GJB2* in 239 Hungarian NSHL patients and the phenotype comparison between homozygous / compound heterozygous and heterozygous cases.

Methods: The total coding region of the *GJB2* gene was analyzed with Sanger or NGS sequencing for 239 patients with NSHL and 160 controls.

Results: Homozygous and compound heterozygous *GJB2* mutations were associated with early oneset serious NSHL in 28 patients. In 24 patients two deletion or nonsense mutation were present with prelingual NSHL, while in compound heterozygous cases with the combination of deletion and missense mutation associated with milder postlingual phenptype. Further 25 cases harbored single heterozygous *GJB2* mutations associated with later oneset, milder NSHL. The most common mutation was the c.35delG deletion, with 12.6% allele frequency.

Conclusion: The mutation frequency of *GJB2* is lower than other European cohort. The most serious cases associated with homozygous and compound heterozygous mutations. In our cohort the hearing impairment and age of oneset was not altered between in cases with only one heterozygous *GJB2* mutations and wild type genotype, which might be exclude the opportunity of autosomal dominant inheritance. In early onset, serve to profound hearing loss cases if the *GJB2* analysis results only one heterozygous alterations further next generation sequencing test is highly recommended. This study was supported by the Hungarian Brain Research Program (KTIA_13_-NAP-A-III/6).

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E-P02.10

A retrospective review of a multidisciplinary hearing loss clinic: 2015-2017

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Background: We established a multidisciplinary paediatric genetic hearing loss clinic for considering genetic diagnoses in children and offering further genetic testing for a subset. Patients were prescreened for GJB2, microarray, imaging, TFT, CMV and haematuria/proteinuria prior to genetics assessment. Funded genetic testing was routinely offered in cases where there was a family history, management or pregnancy implications, or patients could self-fund.

Method: We performed a retrospective review to assess rates of genetic testing and diagnosis from 2015-2017. Outpatient records were reviewed and data, including referral source, ethnicity, family history, inheritance pattern, phenotype, genetic testing, and diagnosis were extracted and analysed for individuals seen in the clinic during 2015-2017.

Results: A total of 90 patients were seen, 22 received a genetic diagnosis and 7 a likely genetic diagnosis (32%). Of these gene panel or exome sequencing was performed in 42 and a genetic diagnosis was confirmed or likely in 17 (40%). Cascade testing was performed in relatives of 17 probands, and demonstrated that 13 cases were inherited. 25 patients had syndromic or likely syndromic causes.

Discussion: This review reports on real world assessment and selection for genetic testing in hearing loss. Clinical prioritisation for genetic testing to maximise clinical utility is appropriate within the public healthcare setting and the application of methods that efficiently optimise outcomes for patients effectively benefit the healthcare system. Our diagnosis rate is higher than previously reported similar studies and demonstrates the clinical utility of NGS testing in a carefully selected subset of patients.

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E-P02.11

Allelic diversity of the *GJB2* gene in deaf patients and ethnically matched controls from Turkic-speaking populations of South Siberia

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Mutations in the *GJB2* gene have the most contribution to hearing loss development in many populations. Spectrum of the *GJB2* mutations and polymorphic variants and their prevalence are highly population-specific. We analyzed allelic diversity of *GJB2* in deaf patients from Turkic-speaking indigenous populations of South Siberia (Tuvinians and Altaians) and in ethnically matched controls. Contribution of *GJB2* pathogenic mutations (c.-23+1G>A, p.V37I, p.R75Q, c.235delC, c.299_300delAT, p.W172C) to deafness was 15.1% in Altaian and 17.5% in Tuvinian deaf patients, according to our estimations. We also evaluated the frequencies of known and new polymorphic *GJB2* variants (p.V27I, p.E114G, p.V153I, p.F192L, p.I203T, c.-23+27G>A, rs5030700, rs3751385, rs9552101, rs117685390, SNP13:20767153C>T) in Tuvinians and Altaians. Variants p.V27I and p.E114G are specific for Asian populations and were found in deaf patients and controls. The p.V27I can be detected as a single variation and also together with p.E114G while p.E114G is almost never found alone. At present, ambiguous association of p.V27I and p.E114G with hearing loss is widely discussed in literature. We proved *cis*-configuration of p.V27I and p.E114G by pedigree analysis and molecular cloning and postulated the presence of allele p.[V27I;E114G] in all cases where p.E114G was found. We estimated frequency of p.[V27I;E114G] in Tuvinian and Altaian controls as 4.78% и 8.68%, respectively, which is higher than in deaf patients (3.93% in Tuvinians, 4.73% in Altaians). Thus, our data confirmed coincidence of p.V27I and p.E114G in *cis*-configuration and absence of association of p.[V27I;E114G] with hearing loss. Study was supported by Project #0324-2018-0016 and RFBR grants (#18-34-00166_mol-a, #17-29-06016_ofi-m).

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E-P02.12

Molecular genetics of autosomal-dominant retinal degeneration in two families suggests novel inherited retinal disease loci

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Introduction: Inherited retinal degenerations (IRDs), among which congenital stationary night blindness (CSNB) and macular degeneration (MD), encompass a large group of clinically and genetically heterogeneous diseases. By using whole exome sequencing (WES) we aimed to identify the disease-causing mutation in two large pedigrees with IRD: one affected by peripheral dystrophy, such as CSNB, and the other with central RD, like MD.

Materials and Methods: Two large pedigrees with an autosomal-dominant form of CSNB (Family 1) and MD (Family 2) were recruited. WES was then performed in 4 individuals from Family 1and in 2 patients from Family 2. Subsequently, single-nucleotide polymorphism microarray and linkage analysis were undertaken on both pedigrees.

Results: Inclusion of rare variants with minor allele frequency (MAF) ≤ 0.5% was performed as an initial filtering step. Sequence variants in genes of the olfactory receptor family and in other polymorphic genes (e.g. mucins) were excluded for downstream analyses since they are unlikely to cause IRDs according to other WES studies. Thereafter, protein coding and splice-site variants were filtered and assessed using in silico prediction tools to prioritize the remaining variants. We firstly searched for mutations in IRD-associated genes. As no IRD-causing mutations were identified in the two pedigrees we performed linkage analysis using autosomal-dominant pattern of inheritance. Haplotype analysis defined different genomic intervals co-segregating with the disease phenotype in both pedigrees and mutation screening of positional candidate genes must be done.

Conclusions: Our results suggest that novel IRD-loci probably exist since no pathogenic changes were found in known IRD-genes.

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E-P02.13

A novel mutation in SCN9A gene involved in congenital insensitivity to pain

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Introduction: Congenital insensitivity to pain (CIP) is a rare condition in which patients have no pain, caused by mutations in Sodium channel protein type 9 subunit alpha (SCN9A) which encodes the voltage-gated sodium channel Nav1.7.

Patient and Methods: A 3 year old girl who suffers from indifference to pain and heat, recurrent episodes of unexplained fever and attention deficient disorder. She has normal growth and development, normal sweat and has tears after emotional events. She was born to healthy no consanguineous Jewish parents from the same diaspora. The mutation was sought by exome sequencing on the DNA of the patient.

Results: Assuming homozygosity of a recessive mutation as the likely cause of the disorder, with allele frequencies of less than 1% in the public databases (ExAc browser, 1000 Genomes and dbSNP). We identified a homozygous variant changing an amino acid in the SCN9A gene with high bioinformatics predictions for damaging effect. The variant segregated as expected in the family members that included 2 unaffected siblings.

Conclusions: Our novel identified missense mutation in SCN9A further expands the spectrum of mutations seen in this gene in association with congenital insensitivity to pain, where most missense mutations cause hypersensitivity to pain. Early identification of the mutation provides early preventive care instructions to the families in handling these patients and contributes for further family planning.

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E-P02.14

A Novel Missense Mutation in *CNNM4* underlying Jalili Syndrome

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Introduction An autosomal recessive syndrome (Jalili syndrome) associating cone-rod dystrophy (CRD) and amelogenesis imperfecta (AI) have been reported in literature. Mutations in *CNNM4* have been described to cause this anomaly. The encoded protein may play a role in metal ion transport.

Materials and Methods: In the present study, a clinical and genetic investigation was performed in a consanguineous family of Pakistani origin, showing typical features of Jalili syndrome. Direct Sanger sequencing was performed in affected and unaffected members of the enrolled family.

Results: Sanger sequencing of *CNNM4* identified a novel missense variant (p.Arg407Leu). The variant is predicted damaging by three in silico prediction tools [SIFT: damaging 0.01; MutationTaster: disease causing; PolyPhen-2: probably damaging 1.000)] and it is not reported in gnomAD. To address the mutational consequences in structure, mutant Arg407Leu was modelled along with the previously reported mutations and deciphered the binding mode of ATP with the aid of docking analysis. Furthermore, molecular dynamics simulations were executed on wild-type and mutant CNNM4 to understand the structural and energetic information after 100ns production run in altering the protein structure, dynamics, and stability. It was examined the conformational shift of ATP binding site in Arg407Leu and binding free energy (MM-GBSA) is lower than the *wt*CNNM4. Additionally, some important hydrogen bonds of Arg407Leu mutant are disrupted during the MD simulations which remained stable in *wt*CNNM4

Conclusion: The present study gives a novel insight into the role of *CNNM4* as a metal transporter in visual function and biomineralization.

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E-P02.15

A case of intragenic PAX2 duplication as a novel cause of renal coloboma syndrome

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Introduction: Renal coloboma syndrome (RCS) involves renal and optic nerve abnormalities. *PAX2* encodes a transcriptional regulator. Biallelic expression of *PAX2* prevents programmed cell death. Clinical features of RCS correspond to tissue-specific expression of *PAX2* during early embryonic development.

PAX2 mutations are identified in approximately 50% of RCS cases and are the only identified genetic cause associated with RCS. Also, ten percent of children with isolated renal hypodysplasia have *PAX2* mutations. Most mutations are missense, nonsense or small deletions/duplications of a few nucleotides in exons 2, 3 and 4 which encode the paired domain. Few duplications of *PAX2* have been reported and have mainly been whole gene duplications. Large intragenic duplications have not been reported in patients with RCS.

Case: A five month old boy presented with a history of renal hypodysplasia and "morning glory" appearance of his optic nerves. He had pre-axial polydactyly and distinctive facial features. Karyotype, analysis for 22q11 deletion and chromosome breakage studies were all normal. No sequencing abnormality of the paired box domain (exons 2,3,4) and octapeptide domain (exon 5) of PAX2 was identified.

Following a renal transplant at 8 years, Array CGH showed a *de novo* 13kb duplication of 10q24.31, resulting in an intragenic gain including exon 5 of *PAX2*.

Conclusion: We describe a child with clinical features of RCS with a novel intragenic *PAX2* duplication. This is a previously unreported pathogenic mechanism for RCS. This case demonstrates the importance of considering dosage analysis investigations when a clinical phenotype is suggestive of RCS.

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E-P02.16

Progressive post-lingual sensorineural hearing loss with unknown etiology in subarctic part of Russia (Sakha Republic)

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Hereditary hearing loss (HL) is genetically heterogeneous sensory disorder. To date more than 140 loci have been mapped and ~ 100 genes were associated with HL. It is known that some orphan genetic diseases being rare in worldwide populations can be accumulated in small isolate populations due to founder effect. We have found 20 cases of progressive post-lingual sensorineural HL (mean age of HL onset 7.22±2.52 years) in indigenous peoples (Yakuts, Evens, Yukaghirs) living in subarctic part of Russia (the Sakha Republic). In 13 out of 20 families we revealed segregation of HL according to autosomal recessive type of inheritance. No any pathology of other organs and systems were found in affected individuals. Speech was preserved in 70% of affected children. However, only 10% of children were able to finish public school while most of patients attended special school for deaf children. Progressive bilateral sensorineural HL of variable degree with onset in the first seven years of life was revealed in affected children while in adult patients (over 18 years) profound deafness was registered. No any pathogenic mutations were found in other genes associated with HL: *GJB2, GJB3, GJB6, SLC26A4, POU3F4* and *12SrRNA* which were previously found in other deaf patients in the Sakha Republic [Dzhemileva et al., 2009; Barashkov et al., 2016; 2018; Pshennikova et al., 2017]. Further studies are necessary for identification of genetic etiology of these HL cases. The study was supported by Ministry of Education and Science of Russia #6.1766.2017, FASO BRK_0556-2017-0003 and RFBR (17-29-06016_ofi_m, 18-015-00212_A).

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E-P02.17

Mutation Screening of Stargardt Disease by Next Generation Sequencing: clinical implication of p. Gly1961Glu (*ABCA4*) mutation

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Introduction: Stargardt disease (STGD1, MIM #248200) is the most common hereditary macular dystrophy affecting children, with a prevalence of approximately 1:10000. STGD is predominantly inherited as an autosomal recessive

trait. Biallelic mutations in *ABCA4* are found in most patients with autosomal recessive STGD (arSTGD) as well as in some patients with autosomal recessive retinitis pigmentosa (arRP) and autosomal recessive cone-rod dystrophy (arCRD).

Patients and methods: In this study, we present two clinical cases of Bull's eye maculopathy with early cone degeneration and the absence of flecks studied by NGS. Genes involved in arSTGD, arRP and arCRD were studied by INGEMM NGS custom panel, Oft-v1.0, designed with NimbleDesign, target bases covered >99%. Bioinformatic analysis was carried out by the Clinical Bioinformatics Unit of INGEMM center.

Results and discussion: Patient 1: *ABCA4* gene, compound heterozygous: p.Gly1961Glu and p. Thr1019Met. Patient 2: *ABCA4* gene, compound heterozygous: p.Gly1961Glu and p.Asn96Thrfs*19. The p. Gly1961Glu mutation in both patients explains the same Bull's eye maculopathy with early cone degeneration and the absence of flecks phenotype. NGS panel in clinical diagnosis permits develop rapid, high-throughput, highly sensitive and accurate testing. Custom panels offer better base-pair coverage, running times, costs and dataset handling than other NGS applications such as whole genome sequencing and whole exome sequencing. Working with custom panels also poses new challenges in variant interpretation, data handling and bioinformatic analyses. To optimize the analyses, panel testing should be performed by bioinformaticians, clinicians and laboratory staff in close collaboration.

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

E-P03.01

Hihg frequency of the severe Q318X mutation in the CYP21A2 gene among Macedonian patients with mild late onset form of 21-hydroxylase deficiency

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Introduction: 21-hydroxylase deficiency is an autosomal recessive endocrine disorder, present as severe salt wasting (SW) or simple virilizing (SV) form, or the milder late onset form (LO). Nine pseudogene-derived point mutations account for about 80% of all defects in the CYP21A2 gene coding the 21-hydroxylase enzyme.

Materials and Methods: DNA samples from 23 Macedonian patients with clinical and laboratory signs of LO form of 21-hydroxylase deficiency, 5 male and 18 female, were collected and subjected to polymerase chain reaction for the detection of presence of nine CYP21A2 point mutations. The patients were evaluated at the Department of Endocrinology and Genetics, University Pediatric Clinic, Skopje, Republic of Macedonia.

Results: Five different mutations were detected in 82.6% (19/23) of the patients on 58.7% (27/46) alleles. The most prevalent mutation was P30L, present in 17 alleles (37%), followed by the Q318X in 7 (15.2%), and V281L, IVS2 and Del 8ntG110 in only 1 (2.2%) allele, each. I172N, cluster exon 6, InsT307 and R356W mutations were not found. In 34.8% (8/23) of the patients complete genotype was revealed (P30L/P30L in 6 patients, Q318X/Q318X and P30L/Q318X in one patient, each). Eleven of the patients were heterozygotes, with no detected mutation on the second allele, and in four patients no one mutation was detected.

Conclusions: We found high frequency of nonsense Q318X mutation, specific for severe classical phenotype of the disease, among Macedonian LO patients. Our founding is comparable to the neighboring Serbian LO population but not with the most of the other European countries.

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E-P03.02

5-Alpha-Reductase 2 deficiency in two siblings with a rare genotype Mirjana Kocova[1], Dijana Plaseska-Karanfilska[2], Predrag Noveski[2], Elena Sukarova-Angelovska[1], Maja Kuzmanovska[2]

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Introduction: 5-Alpha-Reductase 2 deficiency causes DSD in 46,XY individuals characterized with a variable genital phenotype. Determination of the sex of rearing can be complicated. Genetic diagnosis consists of

detecting *SRD5A2* mutations mostly located in exons 1 and 5.

Materials and Methods: We present two siblings with 46, XY karyotype presenting with female external genitalia at birth. Diagnosis was based upon the detection of bilateral inguinal testis in the older child in the neonatal period, and deep voice and Adam's apple enlargement in a second prepubertal child. Measurement of testosterone/dihydrotestosterone was performed. Molecular analysis included multiplex quantitative fluorescent PCR screening for sex chromosome aneuploidies and *SRY* presence, and MLPA analysis for detection of exon copy number changes combined with Sanger sequencing of exons and exons/introns boundaries of the *SRD5A2* gene.

Results: Both patients were compound heterozygotes for two novel mutations in *SRD5A2* gene: c.146C>A (p.Ala49Asp) point mutation in the first exon inherited from the mother, and deletion involving entire first exon inherited from the father. Delayed puberty in the first patient was treated with estrogens with a poor response. Breast implantation and vaginoplasty were performed at the age 20 and 22 years respectively. In the younger sister bilateral orchiectomy was performed with a consecutive slight change in the voice and disappearance of the Adam's apple. The follow up continued up to 22 and 13 years.

Conclusions: Genetic analysis is very useful in precise diagnosis in patients with 5-Alpha-Reductase 2 deficiency. However, final gender assignment is difficult and requires combined medical interventions.

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E-P03.03
Gonadotropin independent precocious puberty and adrenal insufficiency: common clinical presentation of different genetic defects

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Primary adrenal insufficiency can occur at any time during life, including the neonatal period, infancy, and childhood. Children usually present with neonatal salt wasting crisis, failure to thrive with poor feeding, hypoglycemia, vomiting, diarrhea, dehydration, shock, and generalized pigmentation. The development of gonadotropin independent (peripheral) precocious puberty in male children with primary adrenal insufficiency suggest a steroidogenic enzyme deficiency. The most frequent form of congenital adrenal hyperplasia (CAH) is due to a 21-hydroxylase (CYP21A2) defect. We report two young boys with a similar clinical phenotype including early onset primary adrenal insufficiency and gonadotropin independent precocious puberty, both negative for CYP21A2 variants. DNA from the two patients were targeted resequencing using a customized panel of genes involved in adrenal diseases and DSD. A homozygous variant in CYP11B1 and a hemizygous variant in NR0B1 were identified. Mutations of CYP11B1 gene reduce or abolish the activity of 11β-hydroxylase enzyme and cause classical CAH characterized by low cortisol levels and excessive adrenal androgen production starting during fetal life. The typical disorder of puberty in patients with NR0B1 mutations or deletions is represented by hypogonadotropic hypogonadism, but few cases of peripheral precocious puberty, ACTH-dependent precocious puberty and central precocious puberty have been reported. The identified variants have never been reported in literature but homology modeling of the genes demonstrate that both variants affect protein functionality. This study demonstrate as the use of NGS analysis can be decisive in the characterization of cases with apparently similar clinical phenotypes but completely different genetic defect.

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E-P03.04
Role of Rho-quinase polymorphisms in alcohol-induced disorders

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Introduction: Rho kinases (ROCKs) are proteins involved in regulating a variety of physiologic functions including the activation of inflammatory response and hepatic fibrosis through NF-κB signaling pathway. To date, there are no studies showing an association between polymorphisms in rho kinase genes and alcohol abuse or dependence (according to DSM IV criteria) or alcoholic liver disease (ALD).

Here, we have analysed the polymorphisms ROCK 2 rs2230774, ROCK 2 rs978906, ROCK 1 rs35996865 in patients suffering from these conditions in order to evaluate the role of these variants in modulating alcohol abuse or dependence and ALD risk.

Patients and methods: 650 men with alcohol abuse-induced disorders and 220 healthy controls were included in the study. In all cases, DNA was extracted from peripheral blood using phenol/chloroform procedure and genotyped using TaqMan 5'-exonuclease allelic discrimination assays (Applied Biosystems). Statistical analysis was performed using SPSS software.

Results: No significant differences were found in genotype distribution for ROCK2 rs2230774 and ROCK2 rs978906 between patients with alcohol abuse or dependence and control subjects or between alcoholics with liver disease and those without liver damage. However, statistical differences in genotype distribution for ROCK1 rs35996865 were found ($p = 0,05$) comparing patients with ALD versus those without ALD.

Conclusions: Our study does not support the hypothesis that polymorphisms ROCK2 rs2230774 y ROCK2 rs978906 are related to alcoholism or ALD. Nevertheless, ROCK 1 rs35996865 may be associated with genetic susceptibility to develop ALD.

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E-P03.06
Alport as part of contiguous gene deletion syndrome: Further characterization and recommendations

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Alport with diffuse leiomyomatosis is a rare contiguous gene deletion syndrome occurring in 1:1,000,000 in general population.

Aim: To alert for this condition in the differential diagnosis of Alport syndrome, as it might be under-recognized when next generation sequencing panels are used.

Methods and results: Two generation multiplex family is described. The first case was disclosed at age 20y due to leiomyomatosis of her vulva and prior to resection of the excessive muscular tissue. Her examination along with signs of Alport suggested this syndrome on clinical basis. MLPA (research) detected deletion of 250kb consisting of the entire COL4A5 and COL4A6 genes. Further familial evaluation disclosed clinical signs of Alport to her son and daughter with leiomyomatosis. The deletion well segregated in the family and was confirmed by CMA.

Conclusions: Intra familial variability has been detected . As preventable treatment is indicated to both Alport and leiomyomatosis, early diagnosis should be sought as well as PGD or prenatal diagnosis . As several genes causing Alport are currently known, panels using NGS are being offered without del/dup in some. We emphasize the need for seeking this contiguous deletion and the use of appropriate methods for its detection.

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E-P03.07
HLA and non-HLA genetic predisposing factors and environmental effects at Celiac Disease pathogenesis

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Introduction: Genetic predisposition plays a key role in celiac disease. The aim of this study is to investigate the effects of CTLA-4 CT60 A/G polymorphism and environmental factors on Celiac Disease.

Materials and Methods: 45 celiac patients whose HLADQ tissue groups had been known / studied and corresponding number of control subjects were given a questionnaire and a peripheral blood sample was collected for the analysis of CTLA4 polymorphism. CTLA-4 genotype analysis was performed by Sanger Sequencing.

Results: Of the 45 patients, 27 were females with an average age of 11. Of all patients $DQB1_{02}$ homozygosity found in 57% and $DQB1_{02}$ and DQA1 allele homozygosity in 26% . $DQB1_{08}$ allele was negative in 80% and $DQB1_{02}$ DQA1 allele was positive in 86% of patients. Among all celiac patients, 75% were breastfed less than two months, and 45% were introduced to gluten within the first 6 months of birth. The one who were homozygous for DQB1 02 and DQA1 alleles were symptomatic. Abdominal swelling was

less common at the patients with gluten exposure of 2yrs and older ($p = 0{,}042$). Bone pain observed at 66% of 3 allele carriers (DQ2 homozygous DQ_{B1} and positive DQ_{A1}). The CTLA-4 CT60 A/G allele was frequent among controls ($p<.05$).

Conclusion: *W*e determined that HLA DQ allele status effects growth and development at Celiac patients. The HLA DQ2 allele ($DQB1_{02}$ and $DQA1_{05}$) was related to more severe symptoms. The age of gluten exposure is important and CTLA-4 CT60 A allele might have effect on disease etiology.

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E-P03.13

MLPA based identification of compound heterozygous *CYP21A2* mutations in Polish patient with congenital adrenal hyperplasia

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Introduction: Congenital adrenal hyperplasia (CAH) is a group of inherited genetic disorders resulting from a deficiency in one of the enzymes involved in cortisol biosynthesis. In about 95% of cases, CAH is caused by deficiency of the 21-hydroxylase, encoded by the *CYP21A2* gene. More than 100 mutations in the *CYP21A2* gene have been found to cause 21-hydroxylase deficiency.

Materials and Methods: Presented case concerns a 3 year old girl with congenital adrenal hyperplasia. The *CYP21A2* was screened for deletion(s)/duplication(s) and subset of frequent point mutations using the multiplex ligation-dependent probe amplification (MLPA) technique and the SALSA MLPA Kit P050-C1 CAH (MRC-Holland).

Results: MLPA analysis demonstrated the presence of compound two different heterozygous *CYP21A2* mutations which was consist of a large heterozygous deletion of the *CYP21A2* gene and the fragment of pseudogene *CYP21A1P* (the result suggests lower signal for all the probes complementary for *CYP21A2* gene and probes complementary for exons 4 and 7 of the pseudogene *CYP21A1P*) and smaller heterozygous deletion consisting of exons 1-3 of *CYP21A2*.

Conclusions: Congenital adrenal hyperplasia is a common disorder with genetic and clinical heterogeneity. The molecular analysis of CAH is useful in confirming the diagnosis, and provides a powerful tool in genetic counseling. On the basis of the important role of the 21-hydroxylase in the occurrence of CAH symptoms we concluded that this compound heterozygous *CYP21A2* mutation may be a sound candidate for the disease-causing mutation in this patient. In order to determine the inheritance of identified mutations, parental studies are pending.

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E-P03.14

Mutations in the CFTR gene in Bulgarian patients with cystic fibrosis

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Cystic fibrosis is the most common life-limiting genetic disease, which affects many body systems and proceeds with progressive lungs damage. Up to now 2025 mutations have been described in association with the disorder presenting a high allelic heterogeneity of the CFTR gene. According to their effect on the protein they are divided in VII classes. The study of mutations in the CFTR gene in Bulgarian patients has been conducted for more than 30 years. More than 900 patients have been referred to the National Genetic laboratory. The methodology for mutation detection includes SSCP, Sanger sequencing, MLPA analysis and NGS in a small group of a 25 patients with only one mutation. We described 63 mutations in Bulgarian CF patients located in all exons (excluding eighteen) of the gene. Approximately 5% of the molecular defects remained unknown. The distribution of mutations in different classes shows that 12.8% of Bulgarian patients are carriers of class I mutations, 65.3% of class II, 0.4% of class IV, 1% of class V. A part of the mutations (5%) is difficult to be classified, because they are described in single patients and detailed functional studies have not been performed. The determination of the molecular defects played and will continue to play important role for the personalized approach to treating

and achieving a normal lifespan for the patients with cystic fibrosis.

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Genetic markers associated with osteopenia and osteoporosis in patients with cystic fibrosis in Republic of Macedonia

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Introduction: Cystic fibrosis (CF) is the most common autosomal recessive disorder in the Caucasian population which affects most critically the lungs, and also the pancreas, liver and intestine. These patients also frequently have low bone mineral density, osteopenia or osteoporosis. The aim of our study was to determine whether polymorphisms in 4 genes (*VDR*, *COL1A1*, *CTR* and *ESR1*) included in the metabolism of calcium are associated with decreased bone mineral density in patients with cystic fibrosis in Republic of Macedonia.

Material and Methods: Sixty five patients with cystic fibrosis were divided in three groups according to their DXA scans: patients with normal bone density, osteopenia and osteoporosis. DNA was isolated with standard phenol-chlorophorm method of isolation and SSP method was used to determine the polymorphisms in the four genes.

Results: TC genotype of the *TaqI* polymorphism, CT genotype of *Fok1* and AG genotype of *BsmI* polymorphism in VDR gene were the most frequent in all three groups. 23.4% of the patients with normal bone density had the CC genotype, 61.7% CT and 14.89% TT, whilst in patients with osteopenia 10% had CC genotype, 60% had CT and 30% had TT; and in patients with osteoporosis 66.67% had CT and 33.33% had TT genotype in *PvuII* (IVS-397) polymorphism in the ESR1 gene.

Conclusion: Polymorphisms in genes involved in the calcium metabolism are not contributing factor to developing osteopenia or osteoporosis in patients with cystic fibrosis.

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E-P03.16

Role of *CLDN16* gene in one Iranian-Kurdish family with end-stage renal disease

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Introduction: End-stage renal disease (ESRD) occurs when your kidneys clearly begin to shut down. Diabetes, hypertension, and hyperlipidemia increase risk of ESRD. A three- to nine-fold greater risk of ESRD is observed in individuals with a family history of ESRD (Satko SG et al, 2005), so role of genetic study is important now a day.

Material and Method: A 35-year-old, Iranian man who had ESRD with hypercalcemia and hyperoxaluria were detected, and regards to imaging finding medullary nephrocalcinosis was found. According to nephrologist decision, primary hyperoxaluria was considered as one of probably differential diagnosis then who was candidate of kidney and liver transplant. So, genetic analysis was requested to approve mentioned diagnosis. His parents had consanguineous marriage. One brother and one of his sister suffered of same signs and symptoms. Accordingly, to results of whole exome sequencing (WES) and analysis all genes responsible to renal disorder, one likely pathogenic variant (p.C113Y) was found in *CLDN16* gene. This gene is responsible to Hypmagnesia type 3 renal disease with autosomal recessive inheritance pattern. Clinical manifestations in patients fit to Hypomagnesia type 3 renal disease, after reevaluation of clinical data. Familial segregation was done and his parents and one of his sister (they are healthy) are carrier of this variant. It seems organs transplant don't need to do in this case.

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

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Polyposis coli due to a large deletion of the chromosomal Region 5q22.1-q23.1

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We report on an 18 year-old man who presented with recurrent diarrhea and abdominal pain. Colonoscopy revealed classical polyposis coli and additionally adenomas of the duodenum were present. No other affected family members are known. Molecular analysis of the APC-gene showed a de novo heterozygous deletion of the complete APC-gene. In order to check if the loss of one APC copy was part of a greater deletion a microarray analysis was performed and showed a loss of approximately 5.4 Mb in the chromosomal region 5q22.1 – q23.1. Clinically, our propositus did not show any dysmorphic features, but his mother reported about learning difficulties and a developmental delay during childhood. Nevertheless, he was able to attend successfully a regular school and is now working as a craftsman.

Although deletions of several exons of the APC-gene may account for about 15% of classical FAP patients, whole gene deletions are described only in a few patients. In the era prior to MLPA-analysis some patients with cytogenetically visible interstitial 5q deletions have been described with a combined phenotype of polyposis and mental retardation. Our case illustrates, that in case of a lack of biallelic flanking markers in the MLPA-test a microarray should be considered to quantify the size of the deletion. Moreover, this case illustrates the problems that may arise when including a screening for microdeletions in prenatal testing as a first tier test and underlines the necessity of a detailed information of pregnant women prior to testing.

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Familial Mediterranean fever; looking into ten years' experience

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FMF is a hereditary autoinflammatory disorder caused by alteration in Mediterranean fever gene (MEFV). FMF can be seen in all ethnic groups but, it usually occurs in people of Mediterranean origin; Turks Azerbaijanis Jews, Arabs, Greeks, and Italians, Armenians. In general, it is accepted to be autosomal recessive disorder (OMIM # 249100), but there are observed clinical complaints in heterozygous patients, which also suggests the possibility of autosomal dominant (OMIM # 134610) inheritance.Fever, abdominal, chest and joint pain, skin eruption are the most common complaints and AA-amyloidosis with kidney failure is worst outcome. So early diagnosis is essential for improvement of life quality and survival of patients.In this study we correlate the most frequently observed heterozygous and homozygous pathogenic variants with symptoms of patients. In total 4987 patient were analysed for MEFV gene. Patient's data, such as age, gender, consanguinity of parents, complaints, complications, heterozygosity and homozygosity sequence variants were correlated among each other.Pathogenic variants were observed in %52.22 of patients and most common ones were M694V, R202Q, E148Q, M680I and V726A. Most frequent complaints were abdominal pain, arthralgia, fever and chest pain. Consanguinity ratio revealed to be %15.78, but homozygote variants were detected only in %10.8 patients.In conclusion we can say that heterozygous patients also may have a clinical signs and patients with same sequence variants may show phenotypic differences.

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E-P03.23

Locus heterogeneity in goiter and hypothyroidism

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Introduction: The thyroglobulin (TG) gene is organized in 48 exons on human chromosome 8q24. Up to now, 120 inactivating mutations in the TG gene have been identified in patients with congenital goiter and endemic or non-endemic simple goiter. The purpose of the present study is to identify the mutations that cause goiter and hypothyroidism in two members (siblings) of the same family.

Methods: TG gene alterations were identified by Sanger sequencing. Additionally, other genes (TSHR, PAX8, NKX2, FOXE1, DUOX2, TG, TPO, SLC5A5, SLC26A4

IYD, DUOXA2) related with these diseases were studied by NGS.

Results: Both patients inherited the c.2359C>T [p. R768*] mutation from their father, however no alteration was detected in the other allele. The absence of a second mutation in the exonic coding or noncoding (5′ and 3′ UTR) sequences, the promoter region or the exon/intron boundaries, suggested no additional inactivating mutation of the TG gene. Since the single mutation in heterozygosis did not explain the phenotype, further genes related to the disease were studied by next generation sequencing analysis. These studies discovered additional mutations in heterozygosis in genes related to thyroid dysgenesis and dishormogenesis like the c.899-2A>C mutation in PAX8, which affects the splicing process.

Conclusions: These results suggest that several monoallelic defects in different genes which belong to the same pathway could explain the phenotype. However, further studies need to be performed to confirm the cause of the disease in this family.

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E-P03.24
Association of *cagA* positive strains of *Helicobacter pylori* with gastric erosions in Yakut patients (Eastern Siberia, Russia)

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Helicobacter pylori (*Hp*) is a gram-negative, spiral bacterium that colonizes gastric mucosa of human. Clinical outcome after infection related to environmental conditions, immunological factors of host and virulence of microorganisms [Suerbaum et al., 2002]. *Hp* isolates with *cagA*«+» strains are associated with a higher rate of damage and inflammation of stomach, compared to the *cagA*«-» strains [Wu et al., 2003; Hatakeyama et al., 2005]. Clinical outcomes of gastroduodenal diseases depending on virulence and pathogenicity factors of *Hp* is unexplored in Yakut population living in Eastern Siberia (Russia). Our aim was to study association of *cagA* gene presence with erosive gastritis (EG) and chronic gastritis (CG) in Yakuts. We studied *Hp* DNA samples extracted from biopsies of 172 patients with EG (n=77) and CG (n=95). 106 were adolescents (mean age 14.09 ± 2.4 years) and 66 were adults (mean age 41.22±11.84 years). In the group of patients with diagnose EG *cagA*"+" strains was detected in 64/77 patients (83.1%) and *cagA*"-" strains was detected in 13/77 patients (16.9%) ($p<0.001$). Similar data obtained in CG group of patients: *cagA*"+" - 54/95 patients (56.8%), *cagA*"-" - 41/95 patients (43.2%) ($p<0.001$). The high percentage of *cagA*«+» strains may indicate an increased risk development of gastric erosions as well as pre-ulcerous condition. Presence of *cagA* gene is a predictive marker of EG in our patients. The study was supported by the Project of the Ministry of Education and Science of the Russian Federation (#6.1766.2017), by the Project of the NEFU, by the FASO project (BRK 0556-2017-0003).

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E-P03.25
Cys282Tyr, His63Asp and Ser65Cys mutations in 42 patients with hereditary hemochromatosis type 1

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Hereditary hemochromatosis type 1 (HH) is an autosomal recessive disorder of iron metabolism. It is characterized by progressive iron overload and caused by mutation in the HFE gene. The predominant feature of HH is excessive absorption of dietary iron and its deposition in parenchymal tissues and results in cirrhosis, diabetes, skin pigmentation and testicular failure. 42 HH patients (12 females and 30 males) and 106 healthy controls were screened for the Cys282Tyr, His63Asp and Ser65Cys, using polymerase chain reaction amplification of genomic DNA, followed by digestion with Rsa 1 and Bel-1. All patients had the following parameters: iron studies including serum Fe, ferritin and transferrin saturation, serology for hepatitis B and C, liver function tests and abdominal echography . The mean age at genotype diagnosis was 51.3 years in males and 55.3 years in females. 38 from 42 (90.5%) HH patients were homozygous for Cys 282Tyr mutation . Three (7.1%) were compound heterozygous for Cys 282Tyr/His63Asp and one (2.4%) was for Cys282Tyr/Ser65Cys. Five (6.9%) of our controls were heterozygous for Cys282Tyr and one (1.3%) was heterozygous for His63Asp. Hereditary

hemochromatosis type 1 is an underdiagnosed disorder. The most frequent form is associated with homozygosity of the Cys282Tyr mutation. We note that the clinical form in our heterozygotes for Cys282Tyr/His63Asp is much less than that for Cys 282Tyr homocygotes.

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E-P03.26

Progressive familial intrahepatic cholestasis: a study of 6 Tunisian patients

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Introduction: Progressive familial intrahepatic cholestasis (PFIC) is a serious disorder, classified into three types namely PFIC1, PFIC2 and PFIC3, related to mutations in *ATP8B1* gene, *ABCB11* gene and *ABCB4* gene, respectively. The birth prevalence of PFIC is estimated between 1/50000 and 1/100000. Clinical signs of cholestasis in PFIC 1 and PFIC 2 usually appear in the first months of life, whereas the onset of PFIC3 is often delayed. Patients usually develop fibrosis and liver failure before adulthood.

Materials and Methods: We report one patient with PFIC 1, one patient with PFIC 2 and 4 patients with PFIC 3. The molecular genetic analysis was performed in all cases. A total of 167 genes, which have previously been found to be involved in cholestasis disorders, were selected for targeted next-generation sequencing.

Results: Three novel mutations, missense, frameshift and non-sens, were identified in *ATP8B1*, *ABCB11* and *ABCB4* genes. All mutations were found to co-segregate with the disease in the three familial cases.

Conclusion: The molecular study of the progressive cholestasis genes allowed to confirm the diagnosis and then to propose a prenatal diagnosis for future pregnancies. In Tunisia, there are no data on genetic variations in this disorder. This study expands the mutational spectrum of PFIC genes.

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E-P03.27

Bilateral Multicystic Dysplastic Kidney in a three-generation family

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Introduction: Multicystic dysplastic kidney (MCDK) is defined by multiple cystic dilatations replacing the normal renal parenchyma and leading to organ dysfunction. Herein we present a three-generation family with chronic kidney disease (CKD) on bilateral MCDK. **Methods:** The clinical workup was performed for each affected individual. NGS was performed using the Illumina TruSight One Sequencing Panel, on MiSeq Illumina platform for index.

Results: 12years old girl with CKD IIIB diagnosed at age 4 years presents multiple millimeter cysts in bilateral renal parenchyma showed on MRI. The patient's father is on renal replacement therapy with the same renal cystic appearance and associates bifid distal phalanx of one thumb, bilateral sensorineural hearing loss; paternal grandmother is on dialysis with a mild hearing loss and myopia forte. Renal biopsy was yet performed due to parent refusal. NGS results: no pathogenic or probably pathogenic variants were detected (*PKHD1*, *PKD1*, *COL4A5*, *PRKCSH*, *TSC2*, *TSC1*, *EGF*, *TTR*, *FN1*, *UMOD*, *MUC1*, *SALL1* and other genes associated with ciliopathies). Three heterozygous variants were considered as candidates: *RET:*c.C3152T:p.A1051V;*BBS2*:c.C1229G:p.T410,*INPP5E*:c.G304T:p.D102Y, but with no clinical relevance. All three patients and the only sister of our patient's father with no renal impairment present a variant with uncertain significance in *GLA* gene: c.G937T:p.D313Y.

Conclusions: MCKD with autosomal dominant transmission were considered, but the large NGS panel used did not identify any significant variant for the phenotype. Genetic work-up will continue with SNP array for microdeletions/microduplications and whole exome sequencing. Renal biopsy will enlarge the clinical data for a better orientation in molecular analysis and diagnosis.

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E-P03.29

Two novel pathogenic NBAS gene's variants in a patient with infantile recurrent acute liver failure

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Introduction: Acute liver failure (ALF) in infancy and childhood is a life-threating emergency and remains unexplained in about 50% cases. Recently, bi-allelic pathogenic variants in NBAS were reported as a new molecular cause of ALF in infancy, leading to recurrent liver failure.

Materials and Methods: we describe a male patient 2y.5m. of age with episodes of ALF. His infantile period was normal. The first episode of ALF occurred at the age of 1y.9 m. with greatly elevated liver transaminases (ALAT and ASAT more than 1000 U/L), succeeded by sever coagulopathy without jaundice. Alkaline phosphatase was normal, gamma-GT was slightly elevated. Blood amino acids, acylcarnitines and very-long chain fatty acids profile were normal. Organic acids in urine were normal. He had no extrahepatic symptoms. Over 6 months 5 episodes of ALF without fever occurred. After liver crisis, liver function completely repairs and remained normal in the interval.

Results: NGS was used to analyze 47 genes responsible for hereditary diseases with predominant liver damage. Two nucleotide variants in NBAS gene were identified: c.3928 A>G (p.T1310A) and c.4228 A>G (p.T1410A) in compound heterozygous state. Parents and younger sibling are healthy and heterozygous for one or another mutant allele. p.T1310A is benign according to Polyphen2.2 and MutationTaster but located in the conserved region, p. T1410A is possibly damaging and located in the conserved region. ExAc frequency of both substitutions is <0,02%.

Conclusion: Two novel NBAS variants correspond to patient's phenotype and could be the cause of infantile liver failure syndrome-2 in the patient.

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E-P03.30

The CFHR5 nephropathy is not a frequent cause of hematuria in Czech non-Alport families

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Introduction: Complement factor H-related 5 nephropathy is a form of inherited kidney disease. It is endemic in Greek Cypriots, in whom the disease is caused by 6.3 kbp internal duplication in the *CFHR5* gene. This duplication includes exons 2 and 3.

Materials and Methods: The study included eighty patients with microscopic hematuria. We detected no pathogenic variants in *COL4A3*, *COL4A4* and *COL4A5* genes in these patients previously. Thus Alport syndrome was excluded. The *CFHR5* gene was sequenced using sequence capture-based next generation sequencing technology. Copy number variation analysis was also performed. Polymerase chain reaction and 5% polyacrylamid gel electrophoresis were used to detect the Cypriot duplication.

Results: The Cypriot duplication was not detected in any of the patients. Neither pathogenic variants, nor large exonic deletions or duplications were found using next generation sequencing technology in the patients.

Conclusion: The CFHR5 nephropathy is not a frequent cause of hematuria in Czech non-Alport families.

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E-P03.31

Clinical exome sequencing: a new strategy for differential diagnosis of complex phenotypes

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Introduction: In the genomics era, multiple gene investigations by exome sequencing may reveal known or novel variants which are potentially disease causing allowing for effective healthcare management, previously not feasible.

Materials and Methods: A 35-year-old male with a maternal origin family history of renal failure, with unspecified kidney disease, was referred to us for genetic testing. The clinical description was not indicative of a specific gene target therefore, clinical exome sequencing was recommended. Following preparations according to the manufacturer's protocol (SOPHiA Genetics), DNA libraries were sequenced on a NextSeq-500 NGS system (Illumina). Data were analyzed by a bioinformatics pipeline (Sophia

DDM). A gene panel consisting of 101 genes associated with nephropathies was created to filter variants related to the phenotype investigated.

Results: The analysis revealed 3 variants of unknown significance (VUS) in the *PKD1* (c.12817C>T and c.11246G>A) and *ANKS6* (c.1360G>A) genes. Proband's affected and unaffected family members were subsequently tested and the analysis revealed that the two VUS alleles in the *PKD1* gene co-segregated with the investigated phenotype, while the *ANKS6* variant was also found in healthy individuals.

Discussion: Even though further investigation is required to reach definite genetic diagnosis, this case highlights the utility of clinical exome sequencing in clinical practice. Clinical exome sequencing can support definitive diagnosis or clarify differential diagnosis cases in patients with renal diseases of unknown etiology, contributing to precise prognosis, systematic monitoring, and when appropriate, identification of other family members, as well as the possibility of family programming with pre-implantation genetic diagnosis.

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E-P03.32

May being a carrier of CAH lead to phenotypic variability in PAIS?

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Introduction: Androgen Insensitivity syndrome is estimated to be present in 1: 20,000–64,000 male births and variable phenotypic expression. Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD) is an autosomal recessive disorder with impaired synthesis of cortisol and aldosterone and oversecretion of androgens from the adrenal cortex. We present a patient who has a clinical pre-diagnosis of CAH but underwent sequence analysis and he was diagnosed as PAIS.

Materials and Methods: A consultation was requested from the medical genetics clinic for a six-day-old newborn with diagnosis of CAH. Bifid scrotum, hypospadias and pigmentation of the areolae and genital skin were detected in physical examination. Free testesterone and 17- hydroxyprogesterone levels were high. Karyotype analysis was done and then reverse dot blot and MLPA analyses of 21-hydroxylase gene and sequence analysis of the subject's AR gene were performed.

Results: The patient's karyotype was 46,XY. Molecular genetic analysis show a heterozygous mutation c.841G>T of the 21-hydroxylase gene and a hemizygous point mutation c.1174C>T(pPro392Ser)of the AR gene.

Conclusion: Hormonal levels and hyperpigmentation of the patient were misleading in diagnosing. Whether the combination of heterozygous CAH and hemizygous AR mutations may affect the phenotype was discussed by considering the clinical, radiological and biochemical findings of the patient. It was emphasized that PAIS should not be forgotten in the differential diagnosis of patients with minor CAH findings.

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E-P03.33

HLA class II protein and mRNA expression profiles in cultured parathyroid tissues

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Introduction: Allorecognition of antigen presenting cells activated by peptide/human leukocyte antigen (HLA) complex and thus changing its course through lymph nodes where T cells reside.In solid organ transplantation,cultured tissue cells were presumed as passenger-leukocyte free which ensures prolonged graft survival. The aim of this study was to evaluate the potential changes of HLA class II mRNA and protein expression levels during parathyroid cell culture.

Materials and Methods: Parathyroid hyperplasia tissues were obtained from patients who diagnosed with secondary hyperparathyroidism (n=11).After histopathological confirmation,glands were digested using collagenase type II and cultured.Afterwards, cells were collected at day 0(after isolation) and 3,6,9 respectively. HLA class-II (-DR,-DP,-DQα1,-DQα2) antibodies were selected according to binding region and dissociation constant (KD) value which is the equilibrium between antibody and target, then verified

by BLAST. Primers were designed for HLA-DR and -DQ but not for DP. Correlation between protein and gene level results were investigated.

Results: HLA-DR mRNA expression levels remained unchanged, only HLA-DQ mRNA expression level decreased during culture ($p = 0.03$).Protein expression levels of HLA-DP and -DQα2 were higher than -DR and -DQα1 levels during culture ($p<0.0001$).

Conclusions: This study demonstrates that cultured parathyroid tissues are still potential targets for allorecognition even during culture. In addition, -DQα2 and -DR protein expression was found higher in parathyroid tissues.Concordance between DQ and DR indicates a possible linkage in rejection/poor graft survival of parathyroid tissue transplantation via allorecognition. Presented work was financially supported by Bezmialem Vakif University Scientific Research Funding Unit (3.2016/7).

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E-P03.34
Association of PNPLA3 and TM6SF2 variants with alcoholic liver cirrhosis in Serbian population

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Introduction: A recent GWA study identified a missense variant in *TM6SF2* (C>T) and confirmed (C>G) variant in the *PNPLA3* gene as risk loci for alcohol-related cirrhosis. Our aim was to study the association of those variants with the development of cirrhosis in Serbian patients.

Materials and Methods: A total of 103 patients with clinically diagnosed cirrhosis and 103 age and sex matched controls without clinical or laboratory evidence of liver disease were genotyped for *TM6SF2* and *PNPLA3* using PCR-RFLP methodology. Data analysis was performed using IBM SPSS Statistics 21.0.

Results: The prevalence of GG genotype of *PNPLA3* gene was 50% in the group of patients and 21% in control group. We found significant association between GG genotype and cirrhosis (OR 3.8; 95%CI 2.06-6.98; P<0.0001), as well as between G allele and disease (OR 3.09; 95%CI 2.07-4.62; P < 0.0001). There was no significant association between *TM6SF2* genotypes and cirrhosis (OR 1.75; 95%CI 0.82-3.72; P=0.1444), but we observed a significant association between minor T allele of *TM6SF2* and alcohol-related cirrhosis (OR 2; 95% CI 1.05-3.8; P=0.0336).

Conclusions: The variants in *PNPLA3* and *TM6SF2* genes are associated with increased risk of alcoholic liver cirrhosis in Serbian population. Further prospective studies are required to confirm these results and to evaluate the potential of *PNPLA3* and *TM6SF2* as predictors and therapeutic targets in alcoholic liver cirrhosis.

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E-P03.35
Search for a genetic factor for pleuroparenchymal fibroelastosis (PPFE)

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Introduction: Pleuroparenchymal fibroelastosis (PPFE) is an interstitial lung disease characterized by upper-lobe predominant fibrosis. It is a rare idiopathic disease with first symptoms of dyspnea (respiratory distress), dry cough and chest pain due to pneumothorax. Here we present two Turkish consanguineous families afflicted with PPFE.

Materials and Methods: Candidate disease loci in family 1 was detected (LOD score >2.52) by linkage mapping using SNP genotype data of parents and three affected and one unaffected sib. Exome sequencing for one patient from each family was evaluated for rare variants and variants in telomere-related genes (*TERT*, *TERC*, *RTEL1 and PARN*) implicated in PPFE aetiology in some families. Lastly, genes harbouring rare or novel heterozygous or homozygous variants in both patients were evaluated as possible risk factors.

Results: In family 1 we identified novel/rare homozygous variants in *FAM35A* and *TNKS2* and possibly a heterozygous variant in either *FAM22A* or *FAM22D* which are paralogs in a large duplicated region. All variants were predicted as disease causing by computational algorithms. *TNKS2* c.1146A>T segregates with the disease, and *FAM35A* c.540_541insCC will be validated. No candidate variant was found in telomere-related or the common genes with variants.

Conclusion: We had hypothesized that PPFE was a monogenic disease and we would be able to identify the

responsible gene. However, we could not discriminate whether *FAM35A* or *TNKS2* variant is causative. TNKS2 interacts with telomere-related proteins and is highly conserved among species. We hope that our findings would be helpful for future genetic studies on PPFE. (Grant: TUBITAK 114Z829)

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E-P03.36

The contribution of the main genetic marker of type 2 diabetes to the genetic architectonics of type 1 diabetes

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Introduction: A variant of TCF7L2 rs7903146T has the strongest association with type 2 diabetes in most populations and has not the association with type 1 diabetes (t1d) overall. It was shown that TCF7L2 was associated with a subset of children with t1d (with fewer markers of islet autoimmunity) and children with t1d who carried the rs7903146TT genotype were less likely to carry high-risk HLAII genotypes. The aim of this study is analysis of association rs7903146TT and low-diabetogenic HLAII genotypes among t1d children.

Materials and Methods: 249 children (Russian ethnic group) with type 1 diabetes were studied. Allele identification was performed with Real-Time PCR technique. HLA haplotypes DRB1*0301-DQA1*0501-DQB1*0201 and DRB1*04-DQA1*0301-DQB1*0302 were considered high-risk, all others low-risk. The chi-squared was used, and p-value<0,05 were taken to indicate statistical significance of differences.

Results: Carriers of one or two high-risk HLAII haplotypes in the total sample - 56%. The frequency of rs7903146TT genotype in the total sample was 7.3%. Comparison of frequencies of TT genotype in groups stratified by HLAII showed statistically significant differences. 70% of carriers of the TT genotype did not carry high-risk HLAII haplotypes, 10% of carriers of the TT genotype carried two high-risk HLAII haplotype.

Conclusions: The main genetic marker of type 2 diabetes is associated with low-diabetic HLAII genotypes in children with type 1 diabetes. This supports the hypothesis of the possible simultaneous participation of autoimmune and non-autoimmune mechanisms in the development of the disease. This study was supported by the grant № 17-75-30035 of the Russian Science Foundation.

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E-P03.37

Diagnosis of novel splicing defect in the CFTR gene in Russian patient with cystic fibrosis living in Tomsk region

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Introduction: CF is the most common severe inherited disorder, but most CFTR gene mutations are rare. We present here new CFTR mutation in CF-patient from a Russian family, revealed by CF-newborn screening in Tomsk region.

Materials and Methods: We report case of newborn patient with severe CF, pancreatic insufficiency. Different mutation detection methods were combined. The DNA assay for 50 common mutations (Elucigene® CF-EU2v1) and fragment analysis of the CFTR gene fragments were performed as the first test followed by sequencing analysis of the coding and splicing regions of the CFTR gene.

Results: With the Elucigene® CF-EU2v1 assay we identified only one mutant allele p.Phe508del. Analysis of CFTR gene fragments, performed on a capillary sequencer, revealed some abnormalities in peaks profile suggested 5-bp deletion in exon 3. By sequencing analysis of the of exon 3 of the CFTR gene we identified 5-bp deletion mutation in 3'-region of exon 3, which may be due to one of three events in exon-intron junction (according to nucleotide sequence): 1) deletion of Ggtaa - deletion of the last nucleotide in exon 3 and the four nucleotides in intron 3; 2) deletion of gtaag - deletion of 1-5 nucleotides in intron 3; 3) deletion of taagg - deletion of 2-6 nucleotides in intron 3.

Conclusions: New splicing mutation was located in the invariant donor splice site, the mutation name: c.273 +2_273+6delTAAGG or IVS3+2_6delTAAGG. As far as we know this mutation was not been previously described. Identification of mutations has important implications for genetic counselling in families.

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E-P03.38

The association of the TNFSF15 polymorphism rs7848647 and surgical diverticular disease in the Bulgarian population

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Diverticulosis and diverticular disease are one of the most common gastroenterological conditions in the western world. Although believed to be primarily a disease of the elderly, recently there has been an increasing number of cases worldwide, especially in patients under 40 years. The clinical manifestation of diverticular disease can range from mild and uncomplicated to potentially life-threatening inflammation. Recurrent or complicated cases can involve abscess, perforation, fistulizing disease and strictures/obstruction usually requiring surgical intervention. Recent insights into the genetic background of diverticular disease have revealed an association between the polymorphism rs7848647 in the TNFSF15 gene and complicated diverticulitis, requiring surgical intervention. The TNFSF15 gene itself has been associated with other inflammatory bowel diseases such as ulcerative colitis (UC) and Crohn's disease (CD). In this study, 30 Bulgarian patients with various degrees of diverticulitis have been genotyped for rs7848647 in the TNFSF15 gene via Sanger Sequencing. The performed genetic testing has revealed that the most prevalent allele in the selected cohort is the risk allele G, further implicating the role of TNFSF15 as a factor, determining the manifestation and progression of diverticular disease. The results presented here are preliminary and a part of a larger study, aimed at investigating the effects of polymorphic variants within TNFSF15 and their relation to gastrointestinal disease. The increasing number of diverticulosis worldwide prompt for further investigation on the underlying genetic factors of this condition.

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E-P03.39

Case report of a trichohepatoenteric syndrome due to heterozygous compound of novel mutations in ttc37 gen

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Introduction: Trichohepatoenteric syndrome (THES; MIM 222470) is an autosomal-recessive inherited disorder (1:400,000-500,000 live births). THES clinical spectrum comprises distinctive facial features (hypertelorism, broad nasal bridge, prominent forehead), abnormal hair (coarse and fragile with trichorrhexis nodosa), and diarrhea. All affected children require parenteral nutrition to maintain life and growth.

Case Report: Our case is a 1-year girl born to the 37.5 weeks of uneventful pregnancy with weight at birth of 1800 g. At 5 months of age, she began tracking weight stagnation, refusal of food, and increase in the number of bowel movements. She exhibited malnourished appearance, no adipose panniculus, sparse, weak hair and highlights with prominent forehead and hypertelorism dysmorphic facial features. NGS analysis was performed using Illumina (NextSeq 500) technology with a ClearSeq Inherited Disease panel (Agilent Thecnologies), and consisted in the analysis of the coding and intronic regions of SKIV2L and TTC37 gene. Genetic study detected in compound heterozygosity, c.4514T > C (p.Leu1505Ser) and c.3514C > T (p.Gln1172Ter) variants in TTC37 gene. First variant was described as pathogenic in HGMD (CM103463). The variant c.3514C > T is not described in HGMD, ClinVar or LOVD. Prediction in silico suggests that it is a pathogenic change. Detected in 0,0016% frequently population gnomAD (no homozygous); not described in 1000G. Defining the molecular genetic basis of THES will facilitate diagnosis and management. It will help in counselling regarding prognosis and will enable prenatal and preimplantation diagnosis in families at risk and critical insight into the mechanisms of diarrhea and thus treatment.

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E-P03.41

Whole exome sequencing in children with colonic and ileum agangliosis with deafness

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Introduction: The combination of Hirschsprung's disease, hearing loss and pigmentation abnormality are evidence of the Waardenburg syndrome type IV (WS4). It is genetically heterogeneous disease and pathogenic variants finds in the *EDNRB, EDN3* or *SOX10* genes.

Material and Methods: We investigated 3 pediatric patients with the Hirschsprung's disease and the deafness. The DNA-diagnostics had included whole exome sequencing (WES) with following Sanger resequencing, bioinformatic analysis, and cascade familial screening.

Results: Unrelated boy and two girls were operated on the first day of their life in connection with low intestinal obstruction caused by total colonic and ileum aganglionosis. In 2 years all probands had cochlear implantation due to sensorineural hearing loss. Patients don't have skin hypopigmentation, girls are blue eyed blondes. Waardenburg-Shan syndrome was suspected. WES has revealed c.429-1G>A in the *SOX10* gene, p.Pro428Leu and p.Val275Met in the *EDNRB* gene, and c.1280-1281delTG in the *RET* gene. Bioinformatics resources consider them as probably pathogenic. Variants in the EDNRB gene have been found in the parents of the proband, variants in the *RET* and *SOX10* genes are de novo.

Conclusions: All finding variants evaluated as pathogenic with strong or moderate evidence. Mutations in the *RET* gene have described for Hirschsprung's disease, but we didn't find any information that pathogenic variant in the *RET* gene have been found in patients with WS4.

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E-P04 Skeletal, connective tissue, ectodermal and skin disorders

E-P04.02
Chanarin-Dorfman Syndrome: first report of a consanguineous family with all members affected

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Introduction: α/β-hydrolase domain-containing protein 5 (ABHD5) is a lipid droplet-associated protein that promotes the hydrolysis of triacylglycerols (TAGs) by activating adipose triglyceride lipase (ATGL). ATGL is a lipase that catalyzes the initial and rate-limiting step in lipolysis by removing the first fatty acid from TAGs. *ABHD5* gene mutations in humans lead to Chanarin Dorfman Syndrome (CDS), a rare condition in which TAGs accumulate in various tissues, mainly skin and liver. Indeed, CDS is also known as Neutral Lipid Storage Disease with Ichthyosis, an autosomal recessive syndrome characterized by non-bullous congenital ichthyosiform eryhthroderma, hepatomegaly and liver steatosis. To date almost 110 patients have been reported, 27 of them are from Turkey.

Material and Methods: We report here for the first time, the molecular and clinical characterization of an 8 years old patient, born from affected parents.

Results: No particular complications were observed during pregnancy. The child presented non-bullous congenital ichthyosiform erythroderma, hepatosteatosis, hepatomegaly and ectropion. Electropmyographic examination was compatible with myopathy. A cousin of the little patient, born from not affected parents, was diseased too. Leucocytes of patients, stained with May-Grünwald-Giemsa, revealed lipid vacuoles. Molecular analysis showed the homozygous N209X mutation, frequently identified in CDS patients.

Conclusions: to date, the child does not reveal distinctive symptoms or a worsening of clinical traits in comparison with his relatives and other CDS patients. The two children started a diet poor in long chain fatty acids with MCT to improve clinical condition and prevent severe systemic damages.

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E-P04.03
Craniosynostosis as an unusual finding in Noonan syndrome - clinical and molecular description of 3 patients

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Introduction: Craniosynostosis is a result of premature fusion of cranial sutures leading to abnormal shape of head and dysmorphic facial features. In about 15% of cases, craniosynostosis is syndromic and multiple sutures are usually affected. Most of the syndromic patients have a mutation in one of *FGFR* genes, that affect cell signaling e.g. RAS/MAPK pathway.

Patients and methods: We present clinical evaluations of 3 patients with craniosynostosis and the clinical features of Noonan syndrome. The mutation analysis in these patients was performed using classic Sanger sequencing (2pts) or panel next generation sequencing (1pt).

Results: All patients, besides typical features for Noonan syndrome, like short stature, short neck and dysmorphic features, had abnormal shape of the skull. The patient with p.Ala72Ser mutation in the *PTPN11* presented with scaphocephaly, ASDII and cryptorchidism. Patients with mutations (p.Thr58Ile and p.Asp153Val) in the *KRAS* gene had macrocephaly and developmental delay. One of them had parietal foramina, submucous cleft palate and vertebral defect, and the other one choanal stenosis and cryptorchidism.

Conclusions: The fact that the craniosynostosis was observed in our patients with Noonan syndrome caused by mutations of genes of RAS/MAPK pathway confirms the

role of this pathway in skull formation and suture fusion. Clinical follow-up of craniosynostosis patients is recommended to ascertain of additional signs that could help in the diagnosis of non-FGFR-related syndromes.

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E-P04.04
A novel mutation of SALL4 gene resulting in Duane-radial ray syndrome in a Hungarian family

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Introduction: SALL4-related disorders include Duane-radial ray syndrome and acro-renal-ocular syndrome. Duane-radial ray syndrome or Okihiro syndrome is an autosomal dominantly inherited disorder characterized by upper limb and ocular malformations. The disease shows a highly variable phenotype even in the same family.

Materials and Methods: We report a 15-year-old child with radial ray defects: clubhand deformity on the left hand, triphalangeal thumb on the right hand, hypoplasia of the left shoulder girdle. His sister has triphalangeal thumbs and hearing impairment on the right ear. His father has Duane anomaly and hypoplastic right thumb. With international collaboration, Sanger sequencing was performed on the gene SALL4.

Results: A heterozygous deletion of 2 nucleotides SALL4: c.474_475delGA was identified in exon 2 of the SALL4 gene. The variant is a novel variant not previously described in other patients. The deletion causes a frameshift that results in a premuture stop codon (SALL4: p. Glu158AspfsX22). Due to its truncating nature, it was classified as a pathogenic variant.

Conclusions: The above mentioned finding of a pathogenic heterozygous SALL4 variant confirms the clinically suspected diagnosis of Duane-radial ray syndrome. As the father has Duane-anomaly and the sister radial defects, the targeted variant is being tested in our laboratory.

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E-P04.05
A case of infantile systemic hyalinosis associated with a frameshift mutation in the *ANTXR2* gene

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Background: Infantile systemic hyalinosis (ISH) is an autosomal recessive condition characterized by the accumulation of amorphous, hyaline material in the skin and other organs. We report a case of infantile onset systemic hyalinosis due to homozygous mutation c.1073_1074insC in the *ANTXR2* gene presenting as a limb joint stiffness leading to a painful functional disability.

Case: A 2,5-year-old female was monitored since 3 months of age because of limb joint stiffness, and poor weight gain. A patient was born to consanguineous couple from the 1st full-term pregnancy and delivery by C-section with birth weight below 3rd centile. Fetal movements were weak and observed from the 18th week of gestation. Since the age of 6 months she presents multiple painful joint contractures with limited movements, delay of motor development, hyperpigmentation over bony prominences, gingival hyperplasia with impaired eating and failure to thrive. Histopathological examination of skin revealed massive collagenic fibrosis with perivascular fibroblastic reaction. Sanger sequencing of whole coding region of the *ANTXR2* gene identified a homozygous frameshift mutation c.1073_1074insC (p.Ala359Cysfs*13) and in the unaffected parents as heterozygous carriers.

Conclusions: About 50 cases have been described in the literature up to date. This rare disease has been recognised in various ethnic populations, often reflecting presence of consanguinity. Our results support the idea that insertion mutations, causing a translational frameshift, are associated with more severe forms.

D. Brazdziunaite: None. **B. Burnyte:** None. **U. Mickys:** None. **R. Meskiene:** None. **L. Ambrozaityte:** None. **G. Posiunas:** None. **R. Cerkauskiene:** None. **L. Cimbalistiene:** None. **A. Utkus:** None.

E-P04.06
Possible phenotypic expansion of *IFT80*-related skeletal ciliopathy

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Case: This girl had multiple admissions during the first 18 months of life for respiratory compromise requiring ventilator support. At age 6 years obesity and brachydactyly led to a tentative diagnosis of Albright hereditary osteodystrophy which was not confirmed molecularly. At age 2 years, a narrow thorax and wide ribs were noted. At age 6 years, additional skeletal findings included: brachydactyly, short wide metacarpals and metatarsals with cone-shaped epiphyses, bilateral iliac hypoplasia and increased anteversion of the femoral necks. She had near complete lack of enamel of the incisal two thirds of all incisors and canines, and abnormally shaped molars with enamel hypoplasia. By whole exome sequencing homozygosity was detected in the proband and heterozygosity in the parents for NM_020800.2(IFT80):c.1646_1648del p.(Leu549del).

Discussion: Biallelic *IFT80* mutations causing Jeune asphyxiating thoracic dystrophy or short-rib polydactyly syndrome type III have been reported in five individuals including a mesomelic stillborn with bent femurs, trident acetabular roofs, short middle phalanges and metacarpals. Shared features in the others include: a narrow thorax, short ribs, short bent femurs and brachydactyly. Abnormal teeth and obesity have not been described previously in humans. However, studies in mice suggest that *IFT80* may be involved in body weight control and in tooth development.

C.F. Rustad: None. **K. Tveten:** None. **Ø.L. Holla:** None. **Ø.L. Busk:** None. **E. Merckoll:** None. **H. Nordgarden:** None. **I. Mero:** None. **T.E. Prescott:** None.

E-P04.08

Identification of novel mutations in FGFR2 gene in two families with LADD syndrome by Next-Generation Sequencing

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Introduction: Lacrimo-auriculo-dento-digital (LADD; OMIM#149730) syndrome is an autosomal dominant syndrome with variable expression characterized by dental anomalies and digital malformations, hypoplasia, aplasia or atresia of the lacrimal and salivary system with cup shaped ears. Causal mutations have been described in FGF10, FGFR2, FGFR3 and TP63 genes. We present the clinical and genotype applying Next Generation Sequencing (NGS) in two Spanish patients affected by LADD syndrome.

Materials and Methods: Subjects: Patient 1(P1), male of 3 years-old, with of short stature, dental anomalies, alacrimia and auricular dysplasia; father affected of short stature. Patient 2(P2), male of 16 moth-old with auricular dysplasia, dental anomalies and scanty tears; mother affected of ears and dental anomalies. NGS analysis: Libraries and massively sequencing were performed using standard protocol, SureSelect XTPanel Custom(Agilent) in a NextSeq(Illumina). Pathogenic variants were validated by Sanger sequencing and segregation studies.

Results: NGS revealed two heterozygous variants in FGFR2 (NM[[Unsupported Character - Codename]][[Unsupported Character - Codename]]_022970): c.1875_1902del;p.R625fs, deletion that causes a premature stop signal (P1) and c.2165G>C;p.R722T, missense mutation (P2). These variants have not been previously described, but *in silico* prediction software indicated that are found in a highly conserved region with predicted pathogenicity. Both mutations are located in exon 16 in the intracellular tyrosine kinase domain of FGFR2 affecting the tyrosine kinase activity of FGFR2.

Conclusions:1.We present two new mutations, extending the mutational spectrum of FGFR2. 2.Our findings support the implication of FGFR2 gene as responsible for LADD syndrome suggesting a true hot spot for occurrence of mutations in this gene.

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E-P04.09

A rare hereditary connective tissue disease associated with the TGF-β signalling pathway:a case study of Loeys-Dietz syndrome type 1

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Background: Loeys-Dietz syndrome is a rare hereditary connective tissue disease associated with the transforming growth factor beta (TGF-β) signalling pathway. This condition is characterised by an autosomal dominant pattern of inheritance. The global prevalence of Loeys-Dietz syndrome remains unknown. The clinical findings are similar to those of other TGF-β -related connective tissue disorders, i.e., Marfan and Ehlers-Danlos syndromes.

Materials and Method: An eight-year-old male patient was referred to our clinic with a diagnosis of Ehlers-Danlos syndrome. The patient had had previous surgery for craniosynostosis, bilateral inguinal hernia and clubfoot. The patient's physical characteristics included low-set prominent ears, a high palate, dolichocephaly, pectus excavatum, skin-joint laxity, a translucent skin, micropenis and hypospadias. An ophthalmological examination revealed optic atrophy in the right eye. The patient was demonstrated to have aortic and pulmonary artery root dilatation (+3 SD), mitral valve prolapse, scoliosis, dural ectasia, nasoethmoidal encephalocele, enophthalmos and cleft palate on radiological imaging.

Results: The patient was diagnosed with Loeys-Dietz syndrome type 1 as per the clinical and radiological findings. TGFBR1 gene sequencing was performed and p. Ser241Leu(c.722 C>T) heterozygote mutation was detected in exon 4 of the TGFBR1 gene. Mutation was not seen in the parents. Genetic counselling was provided to the family.

Conclusion: Genetic diseases associated with the TGF-β pathway are known to have similar phenotypic effects . It is important to make the correct clinical and genetics diagnosis to ensure early diagnosis and treatment . This case is presented here to increase awareness of differential diagnoses of Loeys-Dietz syndrome.

M. Balasar: None. **M.B. Oflaz:** None. **G.D. Emlik:** None.

E-P04.10

Novel frameshift mutations in fibrillin-1 gene causing Marfan syndrome

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Introduction: Marfan syndrome (MS) is an autosomal dominant hereditary disorder of the connective tissue, which is caused by mutations in the gene encoding fibrillin-1 (FBN1). Up to date, more than 3000 mutations have been identified in FBN1 in relation to MS. Here we report about two novel frameshift mutations found in family and sporadic cases of MS.

Materials and Methods: Family case of MS presented with mother and daughter manifested aortic root dilatation, high myopia, abnormalities of the skeletal system including tall stature and arachnodactyly. Daughter also had lens dislocation. Sporadic case of MS presented with patient exhibited mostly skeletal features of MS with only minor involvement of cardiovascular and ocular systems. The genomic DNA from blood leukocytes of the patients was isolated and the entire coding region and flanking intronic sequences of FBN1 were amplified by PCR. Amplified PCR products were purified and sequenced directly by an ABI 3500 Genetic Analyzer.

Results: Sequencing of FBN1 in mother and daughter with classic MS revealed heterozygous single nucleotide insertion in exon 38: c. 4640_4641insA. This mutation converts threonine to asparagine at amino acid 1547 and terminates translation at codon 1551 (p.Thr1547Asnfs*5). A novel heterozygous one-base-pair deletion of thymidine was found at nucleotide position 5155 in exon 42 (c.5155delT) in sporadic case of MS. This deletion causes a frameshift of amino acids and results in a truncated protein (p.1719Cys>Alafs*174).

Conclusions: We identified two novel frameshift mutations in FBN1. To our knowledge, these mutations have not been reported before in patients with MS.

A. Gusina: None. **S. Miasnikov:** None. **N. Gusina:** None.

E-P04.11

Exon deletions in FBN1 resulting in Marfan syndrome

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Introduction: Mutations in the fibrillin-1 gene (FBN1) cause Marfan syndrome (MS), an autosomal dominant connective tissue disorder. At present, more than 3000 mutations have been identified in FBN1, with the vast majority being single-nucleotide substitutions, small

deletions, and insertions. Here we report about two single-exon deletions found in patients with MS.

Materials and Methods: Genomic DNA samples from 62 patients with suspected MS or MS-like phenotypes were screened using multiplex ligation-dependent probe amplification (MLPA). MLPA analysis was carried out using SALSA kits P065 (MRC-Holland, lot 0506, 0305, 0205) and P066-2 lot (MRC-Holland, lot 0508). Amplification products were run on ABI Prism 310 Genetic Analyzer.

Results: Two gross heterozygous deletions were identified in FBN1. Exon 3 deletion which was previously reported was found in 17-year old male patient with tall stature, dolichostenomelia, arachnodactyly and mitral valve prolapse. Novel exon 49 deletion was detected in 25-year old female patient with classic MS manifested aortic root dilatation, lens dislocation, high myopia, abnormalities of the skeletal system including tall stature, arachnodactyly and pectus carinatum.

Conclusions: We identified two single-exon deletions in patients with MS. To our knowledge, 49 exon deletion has not been reported before in patients with MS.

A. Gusina: None. **S. Miasnikov:** None. **N. Gusina:** None.

E-P04.13

Searching for osteoporosis genes: The use of WGS in an extended Maltese family with osteoporosis

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Introduction: Osteoporosis is a complex metabolic and skeletal disease having a strong genetic background. Indeed, heritability of bone mineral density (BMD) in twin and family studies ranges from 50 to 85%. The aim of the study was to identify known and/or novel genes and gene variants that play a role in the susceptibility of primary osteoporosis in an extended Maltese family.

Materials and Methods: A 2-generation family having multiple relatives with osteoporosis (T-score: <-2.5 or Z-score: <-2.0) at the spine or hip were recruited. Biochemical analysis was performed to exclude other bone diseases. Whole genome sequencing was performed on 12 members and comprehensive filtering strategies were carried out on the single nucleotide variant and indel files. *In silico* modelling and prediction tools were used to determine potential causality of the variants.

Results: Eleven shortlisted variants segregating in a dominant inheritance pattern were identified in the affected relatives having a minor allele frequency of ≤2%. Variants included missense variants within *ADAMTS20* (rs138035327), ARSD (rs78034736), *BMP1* (rs368615556), *CLDN18* (rs114998965), *SELP* (rs754086574), TGFβ2 (rs773943154), TRIM45 (rs146244405), PCDHGA11 (rs138408376), PLEC (rs138924815) and SPARC (rs41290587), and one stop gain variant within WDR89 (rs944955056).

Conclusions: Future studies will evaluate the shortlisted variants by replicating in the Malta Osteoporotic Fracture Study - a case-control collection of more than 1000 Maltese postmenopausal women and other extended Maltese pedigrees so as to determine association with osteoporosis and low-trauma fracture risk at different anatomical sites. Top candidates will in turn be assessed using functional studies.

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E-P04.14

Identification of a new FGRF2 mutation using NGS coupled gene panel testing in Pfeiffer syndrome

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Introduction: The skeletal dysplasias are an extremely heterogeneous group of conditions that affect bone development. They encompass over 400 disorders and most are the result of genetic defects. Pfeiffer syndrome is inherited in an autosomal dominant pattern. It is characterized by the premature fusion of certain skull bones. This early fusion prevents the skull from growing normally and concerns the shape of the head and face. Here we are presenting a case of a 5 months old girl, with healthy parents and with a skeletal phenotype.

Materials and Methods: Total genomic DNA was extracted from her salive sample and analyzed with a comprehensive skeletal dysplasias and disorders gene panel test, that contains the most relevant genes for a skeletal phenotype. We have targeted all of the coding excons with exon-intron boundaries of 186 genes using PCR-based library preparation method. Sequencing reads were mapped to the reference genome (hg19) and after variant calling the variants were classified based on ESP, ExAc, ClinVar and HGMD information.

Results: Gene panel test identified a likely pathogenic heterozygous mutation in the FGFR2 gene (c.940-4_945delCTAGGCCGCC) which encompass the splice site and two codons of exon 8. Currently, this mutation is not

present in the databases, but it overlaps with other mutations, which are connected to Pfeiffer syndrome.

Conclusions: Multi gene panel tests could be very helpful tool in cases, when the patients phenotype overlap with different syndromes. We concluded that, the FGFR2 c.940-4_945delCTAGGCCGCC heterozygous mutation is the cause of the Pfeiffer syndome.

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E-P04.15
yPsychomotor delay in a child with Achondroplasia

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This rare autosomal-dominant disorder achondroplasia (ACh) is caused by a gain-of-function mutation in the gene encoding the type 3 receptor for fibroblast growth factor (*FGFR3*); in more than 95% of cases, the mutation is G380R. Hydrocephalus, a narrow foramen magnum, spinal deformity, and spinal canal stenosis can cause psychomotor delay problems, leading to disabilities in locomotion, communication, and learning. The current study presents the case of a two-year-old male with clinical manifestations suggestive of ACh including, relative macrocephaly, mild narrow thorax, long body, frontal blossing, flatted nose, simian line on the left hand, shortness on the tips of the fingers, and shortness of rhizome in the limbs. Hypotonicity also was presented, he could control his head control, however, he could not sit, turning back without support. He could successfully pick up and hold the objects with his thumb and index fingers. He speaks with words instead of making full sentences. Although a clinical and radiologic ACh has been considered since the admission, there was no cervico medullary compression or hydrocephalus, therefore sequencing analysis carried out to understand the cause of observed skeletal dysplasia due to the predominance of neurological findings. Sequencing analysis has revealed that a heterozygous G1138A mutation within the FGFR3 gene was detected, confirming the diagnosis of ACh. Here we report the first achondroplasia patient with common FGFR3 gene G1138A mutation with psychomotor delay while cervicomedullary compression and hydrocephalus are not presented. We concluded that the neurological manifestations of pediatric patients with Achondroplasia are frequent and very important.

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E-P04.16
Two patients with isolated segmental overgrowth - candidates for PIK3CA gene mutation testing

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We report two unrelated boys with normal karyotype and an isolated segmental overgrowth. This is present together with a 2-3 syndactyly in the 1st-3rd digit of the hand of patient 1 and in the 2-4th digit of the foot in patient 2. In both cases the malformation was present at birth and is progressive. There are no vascular lesions, no dysmorphic features and no delay in psychomotor development. There are no areas of lipoatrophy. The growth parameters (height, weight and head circumference) are between the 75th and 90th centile at the age of 3 years in patient 1 and on the 50th centile at the age of 21 months in patient 2. MRI scan of the affected foot in patient 2 revealed widening of the distal segment of digits 2-4 and a hypertrophy of the adipose tissue. Segmental overgrowth has been shown to result from somatic mutations of the PIK3CA gene and is a feature of CLOVES syndrome and Fibroadipose Hyperplasia. In our patients, Fibroadipose Hyperplasia seems a more likely diagnosis. We plan to perform PIK3CA gene mutation testing on the tissue of the affected foot in patient 2 following a planned operation.

V. Curtisova: None.

E-P04.17
Ischiospinal Dysostosis in a boy with a novel homozygous missense mutation in the *BMPER* gene

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Ischiospinal dysostosis (ISD) is a polytopic dysostosis characterized by minor facial dysmorphism, ischial hypoplasia, short stature with a short spine caused by vertebral anomalies including hypoplasia of the lumbosacral spine,

scoliosis and segmental defects of the cervicothoracic spine and occasionaly associated with nephroblastomatosis. ISD is similar to, but milder than the lethal/semilethal condition termed diaphanospondylodysostosis (DSD), which is associated with homozygous or compound heterozygous mutations of bone morphogenetic protein-binding endothelial regulator protein (BMPER) gene. Here we report on a 3-year and 7 month-old boy with ISD a third child born to a consanguineous couple. He was born at 36 weeks with a birth weight of 1600 gr and delivered via cesarian section. The main clinical findings, high forehead, micrognathia, broad bifid nose, long philtrum, bilateral ezotropia, strabismus, hyperlordosis, short trunk and short stature. ExtremitIes were normal. He is ambulatory, walks with severe hyperlordosis and neck hyperextension balancing his posture. He prefers to bend his body forward when he eat, lookis at objects. He has mild stridor and a hoarse voice, yet nas no tracheostomy. Spinal computerized tomography (CT) showed sacrum agenesis, thoracolumbar lordosis, vertebral cleft formation and posterior fusion defects in low lumbar vertebrates. Kidney CT was normal . Sequencing of *BMPER* gene in the proband revealed the presence of one pathogenic homozygous missense variant c.1166T>G, this variant leads to a p. Val389Gly change in *BMPER*. Despite severe skeletal findings, this ambulatory patient extends the phenotypic spectrum of BMPER-related skeletal disorders.

A. Kablan: None. **B. Mat:** None. **S.G. Temel:** None. **Y. Alanay:** None.

E-P04.18
When short stature leaves you speechless: Floating-Harbor syndrome

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Introduction: Floating-Harbor syndrome (FHS) is a rare cause of syndromic short stature and expressive language delay. To the best of our knowledge 74 molecularly defined cases have been published to date.

Case report: We recently re-evaluated a 10-year-old boy, born at term to healthy unrelated Indian parents. Weight at birth was unknown. Paternal and maternal ages at conception were 33 and 19 years respectively. Main findings in his past medical history were proportionate short stature (-4 to -4.5 SD) with microcephaly (-3.5 to -4 SD) and poor response to GH, nearly absent speech development (2-3 words in Hindi) with normal receptive language and gestures, markedly delayed bone age, delayed motor milestones, bilateral mild-moderate conductive hearing impairment (previous chronic otitis media?), umbilical hernia, surgery for bilateral zonular cataract (at 8 ys), normal echocardiography and brain MRI. On physical examination triangular facies, low anterior hairline, protruding ears, wide nasal base, low hanging columella, short philtrum, wide mouth, thin and straight upper lip vermilion, mandibular prognathia, short neck, brachydactyly, broad fingertips, clinodactyly of the 5th finger of both hands, relatively broad thumbs and halluces were noted. Direct sequencing of the two 3'-terminal exons of SRCAP identified the recurrent de novo mutation p.(Arg2444Ter) (NM_006662.2:c.7330C>T).

Conclusion: We report the second SRCAP-mutated FHS patient of Indian descent. While FHS seems to be very rare based on current literature data, we believe that a low threshold for molecular testing might uncover a significant rate of underdiagnosis worldwide among referrals to tertiary-level centres for short stature.

A.M. Spinelli: None. **M. La Bianca:** None. **E. Rubinato:** None. **A. Morgan:** None. **A. D'Eustacchio:** None. **V. Guastalla:** None. **F. Guidolin:** None. **G. Parmeggiani:** None. **F. Faletra:** None. **I. Bruno:** None.

E-P05 Cardiovascular disorders

E-P05.01
Andersen-Tawil syndrome revealed by next generation sequencing in a patient with Long QT syndrome

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Introduction: Long QT syndrome (LQTS) is characterized by prolonged QT interval because of longer repolarization of the heart after a heartbeat leading to increased risk of tachyarrhythmias that trigger fainting, cardiac arrest or sudden death. Andersen-Tawil syndrome (LQTS7), a form of LQTS, is an autosomal-dominant multisystem channelopathy, characterized by a highly variable triad: periodic

paralysis, ventricular arrhythmias and distinctive physical features. It is incredibly rare, only more than 100 cases have been reported worldwide, about 60% caused by mutations in the gene *KCNJ2* (Type 1) and the rest being unexplained (Type 2).

Materials and Methods: The index patient is a 14 years old boy with LQTS, life-threatening ventricular tachycardia and implanted cardioverter defibrillator. Patient's DNA was analysed by targeted NGS of 176 genes using TruSight Cardio gene panel (Illumina). Variants were validated by Sanger sequencing.

Results: Rare, pathogenic variant was detected in gene *KCNJ2* (Potassium channel, inwardly rectifying, subfamily J, member 2) in chromosome 17: g.68171832C>T, NM_000891.2:c.652C>T, NP_000882.1:p.Arg218Trp. The *KCNJ2* protein product forms a inwardly rectifying potassium channel of crucial significance that regulates cell excitability of cardiac and skeletal muscles. Arg218Trp substitution is within the C-terminal domain of the protein and in heterozygous patients, as in our case, leads to loss of function and dominant-negative effect as previously concluded by functional tests.

Conclusions: Defining the genetic diagnosis may help to distinguish Andersen-Tawil syndrome from other forms of periodic paralysis and prolonged QT interval and furthermore provide appropriate treatment and genetic counselling in affected families.

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E-P05.02
A novel missense *KCNJ2* gene mutation associated with Andersen Tawil Syndrome

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Andersen Tawil Syndrome (ATS) is a rare genetic disorder which is characterized by muscle weakness, ventricular arrhythmias and prolonged QT interval. Among clinical findings are physical abnormalities such as low set ears, short stature, scaliolis, widely spaced eyes and neurocognitive abnormalities such as mild learning disabilities and abstract reasoning.*KCNJ2* gene mutations cause the 60 percent of ATS cases. The protein encoded by *KCNJ2* gene is an integral membrane protein and inward-rectifier type potassium channel. This channel allows potassium to flow into a cell which participates in establishing action potential waveform and excitability of neuronal and muscle tissues. Mutations in the *KCNJ2* gene alter the structure and function of potassium channels changing the regular flow of potassium ions in skeletal and cardiac muscle that can cause periodic paralysis and irregular heart rhythm.In this study, we have performed the cardiac gene-panel sequencing for a patient who has atypical face and limb abnormalities. The patient also has bidirectional ventricular tachycardia and clinically pre-diagnosed with ATS. We identified a novel homozygous missense mutation in *KCNJ2* (NM_000891.2) gene p.V200M (c.598G>A) which is not found in clinical databases such as ClinVar or Human Genome Database (HGMD). In silico analysis of this mutation indicated damaging functional effects. Further in vivo and in vitro analyses will help to establish the causality of this mutation for ATS.

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E-P05.03
Definitive diagnosis of a family with Andersen-Tawil Syndrome (ATS) using targeted clinical exome sequencing

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Introduction: We present a family in whom targeted clinical exome sequencing supported definitive diagnosis of a rare hereditary multisystem disorder, Andersen-Tawil Syndrome (ATS).

Materials and Methods: A boy and his mother were referred to the Department of Medical Genetics, Athens University. Both had similar clinical findings with short stature, dysmorphic features and arrhythmias. Laboratory genetic investigation involved classical karyotype analysis (negative), followed by semi-targeted Exome Sequencing in the proband, using Sophia Genetics Clinical Exome Solution (CES) and Nextera Rapid Capture Exome (Illumina), run on a NextSeq-500 (Illumina). The CES panel includes 4900 genes (114.405 exons). Data was evaluated with two bioinformatics pipelines: *SOPHiA DDM*® (Sophia Genetics) and VarAFT 2.11 (http://varaft.eu).

Results: A known pathogenic mutation p.Arg218Trp was identified in the *KCNJ2* gene, previously reported in ATS.

The variant in both the proband and his mother was confirmed by Sanger sequencing (Chr 17 (GRCh37): c.652C>T, NM_000891).

Discussion: The protein product of *KCNJ2*, Kir2.1, belongs to a potassium-channel family, expressed at high levels in heart, skeletal muscle and neural tissue. Mutations interfere with the function of Kir2.1 in cell excitability, and p.Arg218Trp is located in the domain critical for binding phosphatidylinositol 4,5-bisphosphate (PIP_2), an activator of Kir2.1, whereby weaker channel-PIP_2 interactions reduce current. ATS is characterized by a triad of clinical findings, including muscle weakness (periodic paralysis), arrhythmias and dysmorphic features, although expression is variable. CES can support definitive diagnosis in patients with cardiopathy of unknown etiology, contributing to precise prognosis, systematic monitoring, and when appropriate, identification of other family members.

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E-P05.04
Autosomal dominant inherited *DSP* mutation in an Italian family

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We report the case of a 43 year old woman with an earliest diagnosis of miocarditis. Cardiac MRI detected edema and ephicardic anteroseptal *late gadolinium* enhancement. Clinical evalution confirmed palmoplantar keratoderma (PPK) referred since childhood. PPK were also present in her sister died at the age of 40 for sudden death during physical activity. In order to make a differential diagnosis between miocarditis and desmin-related cardiomyopathy, we performed NGS analysis of a panel of inherited heart-disease genes which identified the pathogenic variant c.6850C>T p.(Arg2284*) in heterozygosis in *DSP* gene. The variant c.6850C>T was previously reported in one individual with ARVD (Arrhythmogenic right ventricular dysplasia) *(Fressart et al., 2010)* and also in a child with Carvajal/Naxos syndromes (CNS) in compound heterozygosis with a pathogenetic variant in *DSP* gene *(Antonov et al., 2015)*. CNS is characterized by woolly hair, palmoplantar keratoderma and cardiomyopathy usually with autosomal recessive and rarely dominant hereditary pattern *(I Keller et al., 2012)*. Proband's son shows PPK and woolly hair. Despite the absence of signs of ARVD in him, our multidisciplinary team agreed to perform a predictive test, especially since he practises athletics at competitive level. Sanger sequencing detected the familial c.6850C>T variant in him. In conclusion, in this family variant c.6850C>T is responsible for ARVD suggesting an autosomal hereditary pattern, excluding miocarditis. We stress the importance of predictive test in young people, even in the absence of cardiac signs, according to the risk that frequent exercise accelerates the development of age-related penetrance and progression to heart failure.

B. Boschi: None. **L. Candita:** None. **E. Contini:** None. **F. Girolami:** None. **U. Ricci:** None. **S. Frusconi:** None. **F. Cutinelli:** None. **A. Marzullo:** None. **M. Targetti:** None. **I. Olivotto:** None. **E. Pelo:** None.

E-P05.06
CTNNA3 gene mutation in a patient withcongenital pulmonary valve stenosis

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ALPHA-T-CATENIN is a cell adhesion molecule coded by the human CTNNA3 gene located on chromosome 10q 21.3. Mutations in this gene are reported in one family with arrhythmogenic right ventricular dysplasia, familial, 13 (ARVD13) also known as dilated cadiomyopathy 13. Its main clinical features are structural and functional abnormalities of the right ventricle with progressive fibro-fatty myocardial replacement and electrocardiographic changes, causing arrhythmias and sudden death. We report here a case of 192 Kb deletion involving the totality of exon 10 of CTNNA3 gene detected by Array-based Comparative Genomic Hybridization using 180 k microarray (Agilent technologies, Santa clara, CA) in one year old male patient presenting a congenital pulmonary valve stenosis (PVS) with mild right ventricular dilation but without arrhythmia or any other malformation. The patient was the result of the third conception of a healthy non-consanguineous spouse. The 1st and 2ed born were a 4 and 2-year-old normal females. PVS is one of the most common types of congenital heart disease (CHD) after cardiac septal defects. The genetic contributors to PVS are not as well defined even if familial forms of non-syndromic PVS have been reported in the literature. Only PTPN11 gene mutations were reported in 50% of cases of PVS associated with Noonan syndrome but never reported in isolated PVS. We suggest here that CTNNA3 gene mutation can be associated with large

phenotype spectrum of CHD including non syndromic congenital PVS.

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E-P05.08

Case report of familial dilated cardiomyopathy caused by LMNA mutation

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Dilated cardiomyopathy (DCM) is an autosomal dominant inherited disease caused by mutations of several genes, among them LMNA. It encodes lamins A/C necessary for functioning and structural integrity of the nucleus. The LMNA mutation carriers have a poor prognosis of life due to rapidly progressive hearth involvement.

We describe a male who developed dyspnea, weakness and abnormal heart rhythm at age of 35. During the next 6 months, heart failure symptoms progressed rapidly. Negative myocardial remodeling and progressive heart failure were observed despite the biventricular resynchronization therapy with optimal medical management, so heart transplantation was accomplished.

We performed NGS using TruSight Cardiomyopathy Sequencing panel (Illumina Inc.) and identified LMNA variant c.565C>T(rs267607626), leading to amino acidic change p.R189W. The variant was indicated as pathogenetic in several predictor programs.

The analysis of pedigrees has been conducted by the Sanger sequencing. The proband and two elder brothers inherited p.R189W from a father who was died suddenly due to DCM at the age of 65. The brothers also passed away abruptly at the age of 28 and 31. Investigation of 25 years-old nephew, who is a p.R189W carrier as well, didn't reveal any cardiac pathology.

We suggest the identified LMNA variant is associated with DCM. The family history reconstruction showed a high prevalence of sudden cardiac death and a wide age range of heart involvement for p.R189W.

D.P. Yermakovich: None. **L.N. Sivitskaya:** None. **T.G. Vaikhanskaya:** None. **A.D. Liaudanski:** None. **T.V. Kurushka:** None. **N.G. Danilenko:** None. **O.G. Davydenko:** None.

E-P05.10

The spectrum of associated congenital malformations in Down syndrome: a retrospective Lithuanian cohort study

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Introduction: Down Syndrome (DS), affecting approximately 1 in 800 live births worldwide, is commonly associated with congenital heart disease (CHD). However, there is no consensus which anomalies are predominant in DS. We investigated DS cohort focusing on congenital heart defects and their associations with extracardiac malformations.

Materials and Methods: A retrospective study enrolled patients diagnosed with DS from 2015 to 2017 at the Department of Human and Medical Genetics, Vilnius University. Data of 65 patients (58.5% males, 41.5% females) was analysed and the major congenital anomalies in infants with DS were examined. Statistical analysis was performed with SPSS Version 17.0 statistic software package.

Results: Trisomy created through meiotic nondisjunction event was the most common cause (93.8%), with the unbalanced translocations and mosaic variants accounting for the remaining cases. The most common associated anomalies were CHD, 42 cases (64.6%), followed by vision disorders (13.8%) and digestive system anomalies (12.3%). The most common cardiac anomaly was patent ductus arteriosus (38.5%), followed by atrial septal defect (35.4%), atrioventricular septal defect (15.4%) and ventricular septal defect (7.7%). 14 children (21.5%) with CHD underwent cardiac surgery within the first year of life. The other common findings were muscle hypotonia, congenital infections and typical dysmorphic features.

Conclusion: We observed a particularly high prevalence of congenital heart defects, vision disorders and digestive system anomalies. The results were similar to findings from other studies. Thus, investigation during the neonatal period and appropriate interventions are essential to improve quality of life and to decrease mortality rates.

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E-P05.11

Hploinsufficiency as a mechanism of *DSP* related diseases

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Introduction: Arrhythmogenic right ventricular cardiomyopathy (ARVC) can lead to sudden death in otherwise healthy young individuals. ARVC is characterized by variable expressivity even within families. 45–50% of ARVC cases are caused by mutations in genes encoding desmosomes, intercellular junctions that anchor intermediate filaments to the plasma membrane, and have both structural and signaling functions.

A broad spectrum of point mutations in *DSP* (desmoplakin), a desmosome component, cause ARVC8 (MIM# 607450) or other OMIM phenotypes (MIM# 605676, 615821, 609638, 612908, 607655) that include at least one of the following: ARVC, cardiomyopathy, tooth agenesis, skin disease (keratoderma, keratosis palmoplantaris, epidermolysis bullosa) or hair defects. Both autosomal recessive and autosomal dominant inheritance has been reported, so it is unclear whether loss of DSP function is the underlying defect.

Materials and Methods: Chromosomal Microarray Analysis (CMA), (Affymetrix CytoScan750K array, Thermo Fisher Scientific, Santa Clara, Ca.) was performed in an amniocentesis sample obtained because of advanced maternal age.

Results: We identified a 286kbp deletion, fully encompassing *DSP*. Family history was found to include a cardiomyopathy-related death, as well as wooly hair in several relatives. The *DSP* deletion segregated with wooly hair in family members. Cardiovascular examination is undergoing to determine its segregation.

Conclusions: This is the first evidence for whole gene deletion of DSP causing an ARVC-related disorder. Our results implicate haploinsufficiency as a mechanism of *DSP*-related diseases, and coupled with previous reports, suggest dosage sensitivity with more severe manifestations in cases with biallelic mutations. Furthermore we recommend CMA testing for unresolved ARVC cases.

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E-P05.12

Novel a-Actin Gene Mutation p.(Ala21Val) Causing Familial Hypertrophic Cardiomyopathy, Myocardial Noncompaction, and Transmural Crypts. Clinical-Pathologic Correlation

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Normal 0 14 false false false IT JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"-Tabella normale"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin:0in; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria; mso-ascii-font-family: Cambria; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Cambria; mso-hansi-theme-font:minor-latin;} A novel a-actin gene (*ACTC1*) mutation is reported as cosegregating for familial hypertrophic cardiomyopathy (fHCM) and left ventricular (LV) myocardial noncompaction with transmural crypts (TC). In an Italian family of nine individuals, four subjects, aged 10, 14, 43 and 46 years, three presenting abnormal ECG changes, dyspnea and palpitation, and one with recurrent cerebral ischemic attack, underwent 2-dimensional echo, cardiac magnetic resonance, Holter monitoring, and next-generation sequencing (NGS) analysis. Two patients with ventricular tachycardia underwent a cardiac invasive study, including coronary with LV angiography and endomyocardial biopsy. In all the affected members, ECG showed right bundle branch block and left anterior hemiblock with age-related prolongation of QRS duration. Two-dimensional echo and cardiac magnetic resonance documented LV myocardial noncompaction in all, and in three a progressive LV hypertrophy up to 22-mm maximal wall thickness. Coronary arteries were normal. LV angiography showed transmural crypts progressing to spongeous myocardial transformation with LV dilatation and dysfunction in the oldest subject. At histology and electron microscopy, detachment of myocardiocytes were associated with cell and myofibrillar disarray and degradation of intercalated discs causing disanchorage of

myofilaments to cell membrane. NGS showed in affected members an unreported p.(Ala21Val) mutation of *ACTC1*. Novel p.(Ala21Val) mutation of *ACTC1* causes myofibrillar and intercalated disc alteration leading to fHCM and LV myocardial noncompaction with TC.<!--EndFragment-->

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E-P05.15
Genetic damage and lipid peroxidation in patients with Hypercholesterolemia

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Introduction: Genetic and environmental factors are important contenders for predisposition to hypercholesterolemia . High levels of low density lipoproteins(LDL-C) may induce an inflammatory response and its atherogenic effects may produce significant quantities of reactive oxygen species,resulting in oxidative stress. This oxidative stress alongwith prolonged medication can induce genetic damage and lipid peroxidation by oxidising the biomolecules.

Materials and Methods: To investigate the state of genetic damage and lipid peroxidation in patients with hypercholesterolemia, a case-control study was carried out. Patients (n=50; 20-40y) from local hospitals and age-, sex- and socioeconomic status- matched healthy controls from general population (n=50; 20-40y) formed the study group under Informed Consent after approval of the study by the Institutional Ethical Clearance Committee. Venous blood samples were used for serum separation and MDA levels (Malondialdehyde:by-product of lipid peroxidation) were determined. DNA damage was investigated in peripheral blood leukocytes of the subjects using comet assay.

Results: Statistical analyses revealed that MDA levels were significantly higher ($p = 0.000$) in patients (2.76 ±0.16 μmol/l) as compared to that in controls (1.45 ±0.08 μmol/l). A significant increase in damage frequency (94.75±4.89;$p = 0.050$), damage index(144.22±107.86;$p = 0.001$) and per cent DNA in tail(44.85±0.89;$p = 0.000$) were observed in patients. Correlation analysis revealed a significant positive association of LDL-C with damage index($p = 0.050$) and MDA($p = 0.000$)and negative association of HDL-C with MDA($p = 0.001$).

Conclusion: High LDL-C levels induces oxidative stress and genetic damage in the patients and thus requires management so as to reduce the risk for cardiovascular and hepatocellular diseases.Financial grant from University for Potential for Excellence is highly acknowledged.

M. Monu: None. **G. Gandhi:** None.

E-P05.16
Hypertension among Italian high school students: genetic and environmental factors. Results from HYGEF project

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Background: Obesity has increased among Italian teenagers predisposing them to hypertension (HT) and cardiovascular diseases. Environmental and genetic factors may account differently in different regions of Italy.

Aims - Enrol 3,000 high school students in 3 regions of Italy (Lombardy, Tuscany and Apulia) in order to:

- determine the prevalence of HT and obesity;
- perform the genetic characterization of some HT candidate loci;
- search for new urinary markers useful for an early renal damage detection.

Materials and Methods: Blood pressure (BP), anthropometric values, a survey on dietary habits of 2960 students and their parents have been collected. Genomic DNAs, extracted from saliva (by OrageneG500, DNAGenotek), have been genotyped by OpenArray Technology (ThermoFisher Scientific) for Adducin and Endogenous Ouabain (ADD-EO) pathway genes.

Results: Regional differences both for estimated sodiuria ($p<0.001$) and SBP values ($p<0.005$) have been observed. BP in the whole sample significantly correlated both with BMI ($p <0.0001$) and sodiuria ($p <0.0001$). Children of hypertensive parents (34%) showed higher SBP values than their peers with negative familiarity ($p = 0.019$).

Subjects carrying mutated variants of ADD1 and ADD2 genes showed greater excretion of urinary Na compared to wild-type ones ($p = 0.015$). LSS gene variants associate to BP. Salt excretion is significantly reduced in subjects carrying a variant of HSD3β2 gene ($p = 0.004$).

Conclusions: The results obtained confirm the role of the genetic network ADD-EO and allow to identify interactions between environmental factors (eating habits) and genetic polymorphisms linked to HT.

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E-P05.17
BIUXX

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The Korean government approved 46 gene and related 12 phenotypes for direct to consumer (DTC) service in 2016. Each of the gene markers is already well known in many previous studies. In this study, we tried to identify the risk prediction of hypertension by the blood pressure marker used in the DTC service based on the DTC service results. We analyzed 647 Koreans for eight blood pressure markers (NPR3, ATP2B1, NT5C2, CSK, HECTD4, GUCY1A3, CYP17A1, FGF5). We took a DNA sample with buccal swap kit and collected the customer questionnaire. We analyzed the results of eight blood pressure markers and age, gender, smoking, drinking, and family history of hypertension in questionnaire. **Among the eight markers, NT5C2 and CYP17A1 showed association tendency with hypertension**. Then, we combined the eight gene marker genotypes and calculated the genetic risk scores based on the number of risk allele and the multiple regression effects size to the hypertension**. As a result, the combined index of the genetic risk score was significantly associated with the questionnaire hypertension history (Odds ratio = 2.72 (95% CI : 1.52 ~ 4.86), P = 0.001). Using this genetic risk score, we estimated the risk prediction accuracy by ROC curve, and the area under cover was 57.4%**. Although there are some limitations, we confirmed that the blood pressure markers based on our DTC service (GeneStyle™) were well selected.

Keywords: Hypertension, genetic polymorphism, DTC

L. Joong Gyo: None.

E-P05.18
Hypoplastic left heart syndrome in a male fetus with 45,X/46,X,i(Y)(p10)/47,X,i(Y)(p10),+i(Y)(p10)/46,XY mosaicism: Do we still need karyotyping?

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Introduction: Chromosomal microarray analysis has significantly increased the ability to diagnose medical conditions caused by copy-number variations. However mosaicism can confound the interpretation of chromosomal microarray results. We describe a case of sex chromosomes mosaic fetus with four cell lines. At the 22th week of pregnancy the hypoplastic left heart syndrome: presence of endocardial fibroelastosis of the left ventricle with aortic valve stenosis, was detected. Because of poor prognosis the pregnancy was terminated. Autopsy of the male fetus confirmed the presence of hypoplasia of the left ventricle and ascending aorta with VSD and mitral valve atresia.

Materials and Methods: QF-PCR test, aCGH analysis using 8x60K array and metaphase chromosome analysis after amniocenteses were performed.

Results: QF-PCR result was normal. Array CGH revealed additional copy of the whole short arm of chromosome Y in approximately 40% of cells and a loss of the whole long arm of chromosome Y: arr(Yp)x2[40],(Yq)x0. Metaphase analysis demonstrated mosaic karyotype: 45,X[82]/46,X,i(Y)(p10)[11]/47,X,i(Y)(p10),+i(Y)(p10)[7]/46,XY[2].

Conclusions: Our data demonstrate that although aCGH should be the first-tier test for clinical diagnosis of chromosome abnormalities, chromosome analysis remain valuable in the detection of mosaicism and delineation of chromosomal structural rearrangements. Phenotype of mosaic subject is primarily dependent on the dominant cell line in a specific tissue. Because our fetus was male we speculated that in gonads the cell lines i(Yp) and XY predominated. The high incidence of hypolastic left heart syndrome is seen in Turner syndrome girls, so the presence of 45,X cell line in our case could explain the heart malformation.

A. Zagorac: None. **A. Golub:** None. **B. Zagradinik:** None. **A. Erjavec kerget:** None. **F. Mujezinović:** None. **R. Kavalar:** None. **N. Kokalj Vokač:** None.

E-P05.19
A new phenotype of severe Dilated Cardiomyopathy associated with a mutation in the *LAMP2* gene previously known to cause Hypertrophic Cardiomyopathy in the context of Danon Disease

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Introduction: Danon disease is a rare X-linked cardioskeletal myopathy with multisystem clinical manifestations. Genetic defects at the Lysosome-Associated Membrane 2 Protein (LAMP2) are the cause of the disorder. Due to the rarity of the disease, there is limited progress in understanding the correlation between genotype and phenotype, and explaining the large variability observed in the clinical features of this disorder.

Materials & Methods: The index case and her relatives underwent full cardiological assessment. DNA libraries were prepared using Illumina's Trusight Cardio sequencing panel, covering 174 clinically relevant genes to cardiac diseases. VariantStudio v2.1 software and Sophia Genetics DDM platform were used for annotation, classification and filtering of genomic variants.

Results: In this study we report two twin sisters, presented in our hospital with end stage heart failure due to dilated cardiomyopathy, requiring heart transplantation evaluation. Genetic analysis showed that they both carried a *LAMP2* missense variant, c.928G>A. The mutation was not detected in their mother. Their father died at 38 years of age, suffering from end stage heart failure of unknown reason. This variant has already been reported by others and was correlated with the clinical triad of Danon disease i.e. hypertrophic cardiomyopathy, mental retardation and peripheral myopathy, as well as autism in one case. In this study, we present dilated cardiomyopathy as a new phenotype for this particular mutation.

Conclusions: The new phenotype of dilated cardiomyopathy associated to the *LAMP2* c.928G>A mutation, presented in this study, confirms the phenotypic heterogeneity of Danon disease.

P. Gourzi: None. **M.P. Pantou:** None. **A. Gkouziouta:** None. **L. Kaklamanis:** None. **D. Tsiapras:** None. **C. Zygouri:** None. **P. Constantoulakis:** None. **A. Chaidaroglou:** None. **S. Adamopoulos:** None. **D. Degiannis:** None.

E-P05.20

Intra- and interfamiliar variability in 4 novel pedigrees with cardiac and neuromuscular phenotypes associated to G382G variant in the LMNA gene

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Introduction: Variants in the *LMNA* gene encoding lamin A/C cause a broad range of different diseases (laminopathies). While clinical features partly overlap, major phenotypic groups include dilated cardiomyopathy (DCM), limb-girdle muscular dystrophy (LGMD), premature aging and lipodystrophy disorders. Genotype-phenotype correlations are tricky but correlations exist between a specific *LMNA* variant and a distinct condition. Less is known about different phenotypes resulting from the same mutation.

Materials and Methods: Four families with multiple members carrying the c.1146C>T (p.G382G) variant in *LMNA* (causing abnormal splicing) were identified. Cardiologic and neurological assessment were performed.

Result: In one family, five individuals carrying the p.G382G variant were affected by a cardiac-only phenotype with DCM and cardiac conduction defects (CCD). Neither the 67-year-old proband had muscular involvement. In three additional families, the index cases (one male and two females) were ascertained for LGMD, with ages at onset varying 2nd-5th decade. Only in the male proband, LGMD was associated to DCM and arrhythmias, while the females aged >60 years, manifested LGMD and CCD. In their families a variable combination of LGMD, DCM, arrhythmias was observed.

Conclusion: We report the same p.G382G variant in *LMNA* associated to different phenotypes within the same and between different families. This unique variant may associate to cardiac-only phenotype, or manifest with neuromuscular involvement. These observations are relevant for genetic counseling and preventive medicine in p.G382G carriers. The mechanisms underlying such variability are currently unknown and their identification would represent an important target in medical genetics.

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Genetic screening of 3 major genes (KCNQ1, KCNH2, SCN5A) in 72 Turkish patients with long QT syndrome: single cardiac center experience

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Long QT syndrome (LQTS) is a genetic disorder characterized by prolongation of the QT interval on electrocardiograms, which may lead to syncope, cardiac arrest or sudden death. LQTS is usually diagnosed after a cardiac event (eg, syncope, cardiac arrest). In some situations, this condition is diagnosed after a family member suddenly dies. Some individuals are asymptomatic; the diagnosis is made incidentally when an ECG shows prolongation of the QT interval. Genetic diagnosis of LQTS is very important for patients and their families because most of the patients are asymptomatic and the clinical follow-up is very difficult. Up to date, mutations in more than 15 genes have been identified in LQTS. Three of these (KCNQ1, KCNH2 and SCN5A) were detected at approximately 75% of cases. Here we investigated for mutations in three major genes responsible for this disease in 72 Turkish patients with LQTS. We detected pathogenic or likely pathogenic mutations in 34 patients. In 28 of our patients, we detected a mutation in the KCNQ1 similar to the literature. We detected a mutation in the KCNH2 in 2 patients and a mutation in the SCN5A in 4 patients. In 7 of our patients, we identified mutations that have not yet been identified in the literature. Although these three gene mutations were reported to be detected at approximately %75 of the LQTS, this rate was %46 in our cases. This suggests that mutations may occur in other rare genes, perhaps even new genes, in our cohort.

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E-P05.22

Targeted resequencing of genes associated with long QT syndrome in Czech patients: two newly identified likely pathogenic variants in previously investigated patient with negative results

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Introduction: Long QT syndrome (LQTS) is a hereditary arrhythmic syndrome characterized by abnormal prolongation of QT interval, increased risk of malignant ventricular arrhythmias and sudden death. With the prevalence 1:2000 it is the most often diagnosed arrhythmogenic disorder. At least 15 LQTS-related genes have been identified so far, nevertheless 75% of mutations are found in 3 major genes (*KCNQ1*, *KCNH2* and *SCN5A*). Here we report identification of likely pathogenic variants in patient previously investigated with negative results.

Materials and Methods: A sequencing panel including 15 genes for LQTS was designed. Genomic DNA was obtained from peripheral blood of LQTS patients or frozen tissue samples for molecular biopsy in cases of sudden unexplained death. Sequencing library was prepared by TruSeq Custom Amplicon kit (Illumina), sequencing was performed on MiSeq (Illumina). Variants in coding and promoter regions were extracted.

Results: We identified 2 variants, Arg562Ser in *KCNQ1* and Phe68Cys in *KCNH2*, in patient where single strand polymorphism analysis (SSCP) was previously performed with negative results. Variant Arg562Ser was previously reported as pathogenic; variant Phe68Cys has not been described yet. Using the ACMG-AMP guidelines both of these two variants are evaluated as likely pathogenic.

Conclusions: Molecular confirmation of diagnosis is important in LQTS cases. It improves the diagnostic accuracy and risk stratification in patients and their relatives. Thus, it is necessary to reanalyze also previously negative patients as new techniques with higher capacity and detection limit are becoming available.

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E-P05.24

Polymorphism rs822396 in *ADIPOQ* is associated to anthropometric, clinic and biochemist alterations related to metabolic syndrome in Mexican population

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Introduction: Adiponectin, encode by *ADIPOQ* gene, is produced mainly by adipose tissue, works as metabolic and immunological regulator. Polymorphism rs822396 in *ADIPOQ* gene was associated with diabetes mellitus II, hypertension and components of metabolic syndrome in Caucasian and Asiatic populations.

Our aim was to evaluate the association of polymorphism rs822396 with anthropometric, clinic and biochemical parameters related to metabolic syndrome in Mexican population.

Materials and Methods: the DNA from peripheral blood of 250 participants was genotyped for polymorphism rs822396 by PCR-RFLP. We obtained clinic, anthropometric and biochemical measures from participants and statistical analysis was made with IBM-SPSSv20.

Results: the analysis was made according polymorphism rs822396 genotype carriers frequency of rs822396G allele in Mexican population was 22%. The rs822396GG carriers (GG/GA) had an increased risk to metabolic syndrome components as body index mass >25 (OR=1.989), glucose >100mg/dL (OR=2.127), cholesterol >200 (OR=1.725), waist circumference (OR=1.995), and triglyceride/glucose index (OR=2.279).

Conclusion: The rs822396 polymorphism of *ADIPOQ* gene could be a molecular marker to alterations related to metabolic diseases in Mexican population.

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E-P05.25
Identification of mutation in *EPHA2* in a family with premature myocardial infarction (MI)

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Coronary artery disease (CAD) and its sequelae myocardial infarction (MI) are the leading cause of death in the Western world. Genome wide association studies have reported many common variants and risk loci that affect cardiac diseases, but they are limited to liking these variants with phenotypic consequences. The importance of genetic predisposition to CAD and MI is best documented by the predictive value of a positive family history. The genetic causes for familial clustering of MI are less clear. Next-Generation Sequencing in families with several affected individuals has revolutionized mutation identification in Mendelian diseases, but can also successfully be applied to more complex phenotypes such as CAD and MI. In this study, we applied whole-exome sequencing and co-segregation analysis in one MI-family and identified a heterozygous c.1324G>T alteration resulting in a substitution of p. V442L in exon 6 of *EPHA2*, a gene located at the 1p36 locus in humans, which is associated with MI. The missense variant, rs772857919 (p. V442L), was predicted as deleterious based on PolyPhe2, deep neural network and MutationTaster. The same mutation was detected in an unrelated patient presenting with MI while absent in the exomes of 2,000 controls. EphA2 receptor plays important role in regulating the cellular events after permanent coronary occlusion and its entanglement in the progression of ischemic cardiomyopathy. Further functional studies are ongoing to understand the underlying pathomechanism. The identification of a mutation in EPHA2 may give us new information about the involvement of ephrin receptor in the mechanics of coronary artery disease.

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E-P05.26
Phenotypic heterogeneity within a family amongst carriers of the same *RBM20* mutation

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Introduction: RBM20 regulates alternative splicing of crucial cardiac genes associated with sarcomere assembly, diastolic function and ion transport. Mutations in *RBM20* have been associated with dilated cardiomyopathy (DCM) with conduction defects.

Materials & Methods: The index case and his relatives underwent full cardiological assessment. Genetic analysis were performed using Illumina's Trusight Cardio sequencing panel.

Results: In this study we present six members of a family carrying the *RBM20* mutation NM_001134363.2: c.1900C>T. The index case was initially diagnosed with DCM at the age of 17 and received an ICD due to ventricular arrhythmias. His brother, carrier of the mutation, is now 28 years old and has been diagnosed with hypertensive cardiomyopathy. The mutation was shown to be of paternal origin, but their father at 54 years old remains

asymptomatic with a mild DCM. The mutation was also detected in index case's aunt who was resuscitated from sudden cardiac death at the age of 48. She had no history of coronary artery disease and echocardiography revealed the initial stages of DCM and a bicuspid aortic valve. Her children were both carriers of the mutation. Her daughter, at the age of 30, was healthy, but her son was implanted with an ICD due to sustained ventricular tachycardia at the age of 21 and presents initial signs of Left Ventricular Non Compaction.

Conclusion: Six carriers of a single mutation in *RBM20* belonging to the same family presented different phenotypes supporting the pleiotropic functional effect of the gene.

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E-P05.27

VDR, _VDBP_ mutations and vitamin D deficiency may cause restenosis in coronary artery disease patients after stent implantation

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Aim: Coronary artery disease (CAD) is a complex and multifactorial disease, can be influenced by pathophysiologic conditions as well as by genetic and environmental factors. Percutaneous coronary intervention (PCI) by balloon angioplasty and stenting have participated in the treatment of CAD. Unfortunately, after surgical intervention, the vascular injury often causes restenosis that can be reduced the luminal diameter of more than 50%. Vitamin D deficiency is a notable risk factor for CAD. Vitamin D receptor (VDR) and vitamin D binding protein (VDBP) gene mutations which play role in vitamin D metabolism may also have role in the progression of the disease as well as restenosis after stent implantation. Therefore, it was aimed to detect *VDR* and *VDBP* mutations and to investigate the relation between other risk factors which may cause restenosis.

Patients and Methods: Ninety three stent implanted CAD patients were enrolled to the study. rs2228570, rs1544410 mutations in *VDR*; rs4588, rs7041 mutations in *VDBP* were investigated by RT-PCR. Other risk factors were also investigated. Results were evaluated statistically.

Results: rs4588 and rs2228570 mutations were found statistically high in patients. Vitamin D deficiency was found statistically significant in patients. Also it was found that there is a relation between myocard infarction and rs7041 mutation. Additionally, rs2228570 mutation was found to be related with vitamin D deficiency.

Conclusion: According to these findings, it was considered that the presence of both vitamin D deficiency and gene mutations which are related with vitamin D metabolism may increase restenosis in CAD patients.

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E-P05.28

Sudden cardiac death (SCD) in the young - value of postmortem genetic analysis in unclear autopsy cases

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Introduction: Cases of sudden unexpected death in young and apparently healthy individuals represent a tragic event for those left behind. A significant number of these cases remain unexplained, even after complete postmortem investigation. Abnormalities in cardiac expressed genes have been associated with arrhythmogenic disorders, which may cause sudden cardiac death. Due to the fact that those diseases are inherited, close relatives of deceased may also be at risk.

Materials and Methods: Postmortem genetic analysis was performed in sudden death cases under the age of 45. DNA was extracted from blood and the samples were sequenced by means of a defined gene panel using Next-generation sequencing (NGS). The sequencing data were subjected to bioinformatics analysis and detected sequence variants were assessed using common databases and applying in silico prediction tools.

Results: In this study, several sequence variants could be identified in the genes analyzed. Due to the detection of numerous unknown and unclassified variants, the interpretation of the results proved to be challenging. However, by means of an appropriate evaluation of the findings, NGS may represent an essential part for the forensic investigation in unclear autopsy cases.

Conclusion: Molecular autopsy is an important tool to support forensic investigation in order to clarify the cause of death and implies great progress for relatives of young SCD victims facilitating adequate risk stratification and genetic counselling. Still, internationally accepted guidelines presenting a standardized course of action need to be established.

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E-P05.29
Evaluation of reports of thrombophilia panel test in women with early pregnancy loss

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Objective: Coagulation disorders are defined as one of the reasons for susceptibility to infertility. Gene mutations that cause coagulation disorders are analyzed with thrombophilia panel test. The aim of the study is to investigate the frequency of gene mutations within the thrombophilia panel test in individuals with early pregnancy loss Materials-Methods: A total of 1538 female patients with early pregnancy loss, aged between 17-54 years who were admitted to the Obstetrics and Gynecology Clinic between 2014 and 2017 and whom mutations were screened in Molecular Genetics Diagnostic Laboratory of Gaziantep University were included in the study. Factor II (G20210A), Factor V (Leiden), MTHFR (C677T and A1298C), Factor XIII (V34L) and PAI-1 (4G/5G) genetic alterations within the thrombophilia panel test were studied by ASO-PCR method.

Results: Homozygous mutation distributions in individuals with early pregnancy loss are; 2%, 0,13%, 11%, 12,8% 0,32% and 23,3% for Factor II (G20210A), Factor V (Leiden), MTHFR (C677T and A1298C), Factor XIII (V34L) and PAI-1 (4G/5G) respectively, and heterozygous mutation distributions are 25,3%, 3,5%, 41,5%, 45,1%, 7,4%, 50,1%.

Conclusion: In conclusion, thrombophilia panel test reports of patients with early pregnancy loss showed that the most frequent homozygous mutation is found as PAI-1 (4G/5G) change and the most frequent heterozygous mutation is also found as PAI-1 (4G/5G) change. Heterozygous mutations were found to be more frequent than homozygous mutations. Assessment of heterozygous mutations in PAI-1 (4G/5G), MTHFR (C677T and A1298C) and Factor II (G20210A) genes in first pregnancy losses will be beneficial for diagnosis and treatment.

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E-P05.30
Serum urate correlates with multiple previously-unexplored cardiovascular proteomic markers

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Introduction: Serum uric acid (SUA) levels are associated with a variety of common diseases, but it is less clear whether it plays a role in the aetiology of these conditions. This project aims to identify correlations between SUA and protein biomarkers for disease.

Materials and Methods: OLINK Proseek panels were used to measure serum levels of biomarkers for inflammation and cardiovascular disease in the CROATIA-Vis and ORCADES cohorts (combined n=1,470). 266 proteins were included in the analysis. Spearman correlations were calculated between SUA and protein biomarkers, along with 20 additional phenotypes. Measurements were corrected for kinship, age and sex. To account for confounding between phenotypes, partial correlations were calculated between SUA and each phenotype. Additionally, multiple lasso regressions were run and each phenotype scored on how often it was retained in a predictive model for SUA.

Results: Significant partial correlations were detected between SUA and Fibroblast Growth Factor 23 (FGF23), Epithelial Cell Adhesion Molecule (EpCAM) and Insulin Like Growth Factor Binding Protein 2 (IGFBP2). Partial correlations were calculated for these three proteins, creating a network model of correlations that provides

context for their relation to uric acid. These associations have persisted in sensitivity analyses, and have also been detected using lasso regression. Replication of the results in independent cohorts is in progress.

Conclusions: Uric acid correlates with multiple cardiovascular disease and inflammation biomarkers. Investigation into the genetic basis of these associations may help better understand the debated role of SUA in cardiovascular disorders.

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E-P05.31

Expression of angiogenic factors VEGFA/VEGFR2 and chemokines SDF-1/CXCR4 in spontaneous abortions

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A series of studies have reported that increased concentrations of pro-inflammatory or T helper cell cytokines or increased frequencies of natural killer (NK) cells in the blood can be found during euploid sporadic miscarriage. There is some evidence that uterine NK cells regulate angiogenesis in the endometrium and therefore may also play a role for implantation and early pregnancy. NK cell density was positively correlated with the formation of blood and lymphatic vessels, spiral arteriole smooth muscle differentiation and oedema in endometrium of women with recurrent pregnancy loss. In our study, by using real time PCR technique, we measured the expression levels for the most important angiogenic factors VEGFA and its receptor VEGFR2, as well as for chemokine SDF-1 and its receptor CXCR4, in decidua samples from spontaneous abortions compared to decidua from matched controls of elective abortions. Six endometrial samples from euploid sporadic miscarriages and 4 samples from matched controls were investigated for mRNA levels of these angiogenic factors and chemokines. The results showed increased expression of all studied molecules: about 3 times average increase in the expression levels of VEGFA and CXCR4, as well as higher increase in the expression of SDF-1 (by 7 times on average) and VEGFR2 (by 9.8 times on average). Our results suggest dysregulation of VEGFA/VEGFR2 angiogenesis in the endometrium of miscarriages, along with the higher expression of CXCR4 (chemokine receptor, expressed by NK cells) and its chemokine-ligand SDF-1 (most probably expressed by endothelial cells).

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E-P05.32

Patient with recurrent ventricular fibrillation and polymorphic nonsustained ventricular tachycardia with mutation in MYLK2 gene

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Introduction: Ventricular tachycardia (VT) and ventricular fibrillation (VF) are frequent clinical arrhythmias in patients with hypertrophic cardiomyopathy (HCM). We are reporting a case of young patient after VF without familiar history of sudden cardiac death (SCD) or structural heart disease, with still undescribed variant in MYLK2 gene detected by NGS. **Material and Methods:** Patient was 37 years old women after SCD and CPR procedure due to VF. On admission patient was stable and conscious. Blood tests and echocardiography were normal, on ECG a sinus rhythm was present. Coronary angiography and electrophysiologic testing were both normal, only pair of ventricular premature complexes (VPC) from septal region were observed. Several polymorphic VPC triplets were present on Holter. Patient was declared idiopathic VF and an ICD was implanted. Up to now two episodes of VF and several episodes of polimorphic VT were treated with ICD.

Results: NGS genetic testing using TruSight Cardio (Illumina) was performed, mutation NM-033118.3: c.4G>A in MYLK2 gene was detected.

Conclusion: MYLK2 gene mutations are present in some familial HCM. Since our patient presents no signs of cardiomyopathy or structural heart disease, it is possible that clinical form of HCM will evolve later. Detected variant of c.4G>A in MYLK2 gene is currently classified as a variant of unknown significance but due to highly arritmogenic and malignant clinical course of disease in present case it is wort to describe it. The work was done in the framework of an internal research project of UKC-MB: IRP-2015/01-07.

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E-P06 Metabolic and mitochondrial disorders

E-P06.01
NGLY1 mutation in three siblings; a case report and literature review

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Congenital Disorder of Deglycosylation is a group of disorders with defect in glycosylation[1]. It is an autosomal recessive disorder with low or absent tear production, low muscle tone, unusual muscular jerks and psychomotor retardation. Additional features are microcephaly, intractable seizures, and evidence of liver dysfunction.

We describe a family with three affected offspring. The thirty-year- old male suffered from hypotonia, bent posture, muscle contractures, muscle atrophy, abnormal muscle movements during sleep. His 34-year old brother had the same symptoms plus more significant abnormal muscle movements, constipation and right-sided scoliosis. The 35-year old sister had the same symptoms in addition to strabismus. All three cases had normal tear production, normal head circumference,and no seizure. Isoelectric focusing was normal in all three siblings. Liver enzymes and ultra-sound examination of liver was performed in one and was normal. Parents are first cousin and there is no similar case in family. Next Generation Sequencing for Intellectual Disability was performed identifying a homozygote pathogenic variant defined as c.708G>T (p. Trp236Cys) in exon 5 of NGLY1 gene. Both parents and siblings were also checked by Sanger Sequencing and were heterozygout. The clinical findings are not similar to what has been reported to previous patients with NGLY1 mutations and present new clinical features such as contractures and muscle atrophy. Here we expand the clinical phenotype associated with NGLY1variants.

1.Ennns, G. M., Shashi, V., Bainbridge, M., and 31others. Mutations in NGLY1cause an inherited disorder of the endoplasmic reticulum-assosiated degradation pathway. Genet. Med. 16:751-758,2014.Note:Erratum:Genet Med. 16:568 only, 2014.[pubmed:24651605]

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E-P06.02
Menkes disease: a case report of patient with de novo novel variant in ATP7A gene

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Menkes`s disease (MD) is an rare X-linked recessive disorder with systemic copper deficiency caused by pathogenic variants in ATP7A gene, encoding a copper transporter P-type ATPase. MD is characterized by an extensive clinical heterogeneity, as a direct consequence of dysfunction of several copper-dependent enzymes. Severe progressive neurodegeneration, seazures, developmental regresion and „kinky“ hair are typical characteristic of clasical MD. Over 200 different mutations, affecting ATP7A, gene have been reported with one-third of cases arise from de novo mutations.

We present a 13 months old boy with MS, who appeared healthy until age of six months, when loss of developmental milestones, hypotonia, seizures, and failure to thrive occurred. Clinical synopsis comprised microcephaly, temporal bossing, poor facial and body movements, “kinky”, “steely” hair, pale, eczema skin, pectus excavatum, trunk hypotonia, spasticity of extremitas and severe mental retardation. Decreseed levels of serum copper and ceruloplasmin, metaphyseal flaring in the long bones, bladder diverticula, focal cortical reduction and defective myalinization and pili torti were revealed. Molecular genetic analysis (DNA sequencing, MLPA) of the ATP7A gene revealed the hemizygous ATP7A mutation c.3226delA (p. Ile1076Leufs*13), not detected in the mother of the patient. This „de novo“ alteration in ATP7A gene has neither been described in the literature (www.hgmd.org), but the nature of the alteration (frameshift with premature stop codon) indicates the mutation is with very high probability pathogenic.

A typical clinical picture of the MD in the reported patient with the novel variant c.3226delA in ATP7A gene could contribute to better understanding of genotype-phenotype correlation in MD.

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E-P06.03
When mitochondria hide among the tiger stripes

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Introduction: *COXPD12* (LTBL – leukoencephalopathy with thalamus and brainstem involvement and high lactate) is an autosomal recessive mitochondrial neurologic disorder, caused by mutations in the *EARS2* gene. The condition is characterized by early onset hypotonia and delayed psychomotor development, a specific MRI finding – leukoencephalopathy affecting primarily the deep white matter, brainstem and cerebellum. Serum lactate levels are typically elevated due to defects in mitochondrial oxidative processes.

Clinical case: We present a 4 year old girl with severe psychomotor deficiency, mild dysmorphic features, a number of seizures with different clinical repertoire and normal serum lactate. A brain MRI was performed, showing a „tigroid pattern" in the deep white brain matter. The following potential explanations were eliminated during the diagnostic investigation: metachromatic leukodistrophy, saposin B deficiency, Pezilaeus-Merzbacher disease, autosomal recessive spastic ataxia of Charlevoix-Saguenay and Krabbe disease. Finally, molecular-genetic testing revealed a pathogenic mutation in the *EARS2* gene which established the correct diagnosis – COXDP12.

Results:

Gene sequence	Variant	Variant ID	Primary transcript changes	Aminoacid sequence changes	Variant type	Zygosity
EARS2	Chr.16: g.23555998G>A	Rs376103091	NM_ 001083614.1: c.322C>T	Arg108Trp	Missense class 5 - Pathogenic	Homozygous

Conclusions: A pathogenic variant of this missense mutation causes replacement of arginine with triptophan at 108th position in the aminoacid sequence encoded by *EARS2* gene. This explains the majority of clinical manifestations, however the lack of increased lactate levels, as well as the tigroid brain pattern make this case intriguing in matter of widening the phenotypic spectrum of the disease.

Key words: *COXDP12*, LTBL, leukoencephalopathy, *EARS2* gene, psychomotor deficiency

R.S. Tincheva: None. **T.N. Delchev:** None. **H.M. Kathom:** None. **D.M. Avdjieva-Tzavella:** None.

E-P06.04

Novel compound heterozygous pathogenic variants in the *CPS1* gene in a newborn with Carbamoyl Phosphate Synthetase 1 Deficiency

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Carbamoyl phosphate synthetase 1 (CPS1) deficiency is a disorder of the proximal urea cycle. It is an autosomal recessive disorder which presents with severe hyperammonemia, typically in the newborn period. We report a case of a boy with CPS1 deficiency who presented at day 3 of life with decreased consciousness and shallow breathing.

Diagnosis: He had severe metabolic acidosis with hyperammonemia(813umol/L). Biochemical investigations showed raised glutamine (3984umol/L), low citrulline (<5umol/L) and normal urine orotic acid(4.0umol/L), suggesting a diagnosis of a proximal urea cycle defect (UCD). Targeted DNA sequencing of *CPS1* and *NAGS* genes revealed that patient was a compound heterozygote with 2 novel pathogenic mutations in the *CPS1* gene: c.3241del, p.(Leu1081fs) in exon 26 and c.3966C>A, p.(Asp1322Glu) in exon 33, confirming the diagnosis of CPS1 deficiency.

Interventions: High dextrosity drip with intravenous lipids to promote anabolism and prevent catabolism, ammonia scavangers such as sodium benzoate and sodium phenylbutyrate, supplementation of essential amino acids with arginine, citrulline, and empirical trial of n-carbamylglutamic acid. Removal of ammonia was also facilitated by hemodialysis.

Outcomes: Despite optimization of above therapy, ammonia levels remained high and decision was made with parents for a one-way trial off dialysis. Ammonia levels continued to rise and the child was discharged home for palliative care at 20 days of life. He passed away at 22 days of life.

Lessons: Measurements of biochemical intermediary metabolites are insufficient to differentiate between CPS1 deficiency and N-acetylglutamate synthetase deficiency, but these results should guide targeted DNA sequencing of UCD to facilitate management and genetic counseling.

N.W.Y. Fong: None. **S.S. Jamuar:** None.

E-P06.06

Gene therapy - a practical approach: cloning the LDLr gene in an attempt to treat hypercholesterolemia

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Gene therapy has been a great promise in modern medicine for many years. The current presentation is focused on a personal research in the field, which took place at Aarhus University, in Denmark, under the supervision of Professor Thomas G. Jensen. In an attempt to cure and/or contribute to the treatment of hypercholesterolemia cases, the experiment started having a specific target: obtaining gene expression of the cloned LDLr gene within keratinocytes. All steps were perfomed within this experiment, starting from cutting off the gene sequence from human DNA (previously isolated from hepatocytes) using Eco RI, obtaining a construct of the gene fragment and lambda phage (called GCSam-LDLr) as a vector that also included a plasmidic sequence of resistance to neomicin, followed by ligation and transformation into a circular, single-stranded chromosome of E.Coli. Many control points were set and used during all these steps (using several restriction enzymes: Bam HI, Hind III, Eco RI, Xho I, Xba I, Not-1 and Sal-1). The E.Coli colonies were developed and selected using the new bacteria's resistance to neomicin. The last step was the "infection" of keratinocytes with the transformed bacteria. Gene expression was obtained in a spectacular proportion.

T.T. Grozescu: None. **T.G. Jensen:** None. **L.C. Bohiltea:** None.

E-P06.08

Characterization of a novel GPIHBP1 large deletion in an infant patient with hypertriglyceridemia

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Characterization of a novel GPIHBP1 large deletion in an infant patient with hypertriglyceridemia

Type 1 hyperlipoproteinemia is an autosomal recessive disorder characterized by severe hypertriglyceridemia, recurrent episodes of acute pancreatitis, lipemia retinalis, and cutaneous eruptive xanthomas. Loss-of-function mutations of GPIHBP1 have been reported as the cause of type I hyperlipoproteinemia in several patients. In this case report, we characterize a novel 3,8 Kb homozygous deletion comprising exons 3 and 4 of GPIHBP1.

A newborn male of Pakistani origin who presented congenital hypertriglyceridemia in a consanguineous family was tested for germline mutation in APOA5, APOC2, GPIHBP1, LIPI, LMF1 and LPL genes by Sanger sequencing. No mutations were detected in any of these six genes, but no PCR products were obtained for exons 3 and 4 of GPIHBP1, so this patient could be homozygous for a deletion comprising exons 3 and 4 of the GPIHBP1 gene.

To verify and characterize the deletion harbouring exons 3 and 4 in GPIHBP1, several long-range PCRs were carried out by combining a forward oligo in exon 2 and different reverse oligos in 3'UTR, and the deletion breakpoints were finally characterized. Sanger sequencing confirmed the presence of a 3,8 Kb homozygous novel deletion in GPIHBP1: NM_178172.5:c.181+509_*2768del3828 – Chr8(GRCh37):g.144296334_144300161del.

E. Del Nuevo Martinez: Other; Significant; LabGenetics. **R.B. Fisher:** None. **J. Puente-Prieto:** Other; Significant; LabGenetics. **A. Sesto Yague:** Other; Significant; LabGenetics.

E-P06.09

Two Different Genetic Diseases in the Same Patient

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Introduction: Rare disease is defined as a condition that affects fewer than 200,000 people. The consanguinity increases the risk of cooccurence of two etiologically different recessive Mendelian diseases in a single family.

Materials and Methods: Here we represent a Turkish family with two affected children who had different clinical findings. The affected boy was born as the first child of consanguineous family and died at 8 years old. The affected girl was born as the third child of the family and is 5 years old.

Case 1: He was diagnosed as Leigh syndrome clinically. He had hypotonia,mental and growth retardation, movement poblems

MRI findings: hyperintensity compatible with Leigh disease

Case 2; She has hypotonia, nystagmus, strabismus, growth retardation, delayed language development

Biochemical tests: Elevated plasma concentrations of branched-chain amino acids, ketonuria

Cranial MRI: hyperintensity in basal ganglia

Results: WES analysis revealed homozygous likely pathogenic variant in the DUBT gene and homozygous VUS variant in the NDUFS7 gene, in case2. Both parents are heterozygous carriers of the each detected variants. DUBT mutations cause Mapple Syrup Urine Disease and NDUFS7 mutations cause Leigh syndrome.

Conclusions: In some families different clinical findings may due to existence of more than 1 gene defect. Our report demonstrated that such coexistence of different diseases in

same family should be expected when analyzing rare diseases in consanguineous families. Our report emphasize that the presence of two rare diseases in one family should be taken into account when the presence of broad spectrum of phenotype and consanguinity..

L. Özer: None. **E. Unsal:** None. **S. Aktuna:** None. **V. Baltacı:** None.

E-P06.10

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Introduction: Neonatal mitochondrial encephalocardiomyopathy (OMIM, 614052) is due to a mutation of transmembrane protein 70 gene (TMEM70). TMEM70 encodes a mitochondrial membrane protein that plays a role in the biogenesis of mitochondrial ATP synthase.

Materials and Methods: The authors report a boy aged 1 year and 9 months who presented immediately after birth with signs of encephalopathy associated with lactic acidosis, severe hypotonia, hyperammonemia, and 3-methylglutaconic aciduria. The infant was also found to have hypertrophic cardiomyopathy, atrial septal defect, pulmonary hypertension, hypospadias, moderate psychomotor developmental delay. It is the fifth child in the family, born prematurely, low birth weight (1900 g); consanguineous family of Roma (Gypsy) ethnic origin.

Results: Genetic testing revealed a homozygous mutation (c.317-2A>G) in the TMEM70 gene. This mutation is prevalent, particularly in the Roma population. The inheritance is autosomal recessive. The prevalence of the syndrome is unknown.

Conclusion: To date fewer than 100 cases have been reported in the literature (Orphanet). This is the only case reported in Romania.

Keywords: mitochondrial encephalocardiomyopathy, TMEM70, ATP synthase

A.D. Jurca: None. **M. Bembea:** None. **K. Kozma:** None. **C. Petchesi:** None. **A. Szilaghy:** None. **A. Balmos:** None. **D. Dubau:** None. **C. Jurca:** None.

E-P06.11

A novel *MYT1L* mutation in a patient with severe early-onset obesity and intellectual disability

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Introduction: The genetic and molecular mechanisms underlying severe early-onset obesity are still incompletely understood. Deletions at 2p25.3 have been reported in several patients with obesity and intellectual disability. *Myelin-transcriptor-factor-1-like* (*MYT1L)* gene in this locus has been proposed a candidate gene for obesity. MYT1L is expressed in the developing brain and has been recognized as part of the leptin-melanocortin-SIM1 pathway.

Materials and Methods: We report a 13-year-old boy presenting with overweight already at 1 year of age (body mass index (BMI) Z-score +2.3) and obesity at 2 years of age (BMI Z-score +3.8). The patient had hyperphagia, delayed neurological, cognitive and motor development. He also had speech delay, strabismus, hyperactivity and intellectual disability. Brain MRI was normal. The parents and sister had normal BMI.

Results: Whole-genome sequencing, including DNA samples for the index and his parents and sibling, identified in the index a novel heterozygous *de novo* frameshift deletion that introduces a premature termination of translation NM_015025.2(MYT1L): c.2215_2224delACGCCTGCC, p.(Thr739Alafs*7) in *MYT1L*. The frameshift variant was confirmed by Sanger sequencing.

Conclusion: Our finding supports the association of *MYT1L* mutations with early-onset syndromic obesity. The identification of novel monogenic forms of childhood-onset obesity will provide insights to the involved genetic pathways. Further studies on MYT1L will increase our understanding of its biological function, role in appetite regulation and development of syndromic features, and may identify potential targets for therapy.

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E-P06.12

New *NDUFS6* variants are associated with Leigh syndrome and cause multiple deficiency of OXPHOS complexes and complex I assembly defect

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The genetic causes of Leigh syndrome are heterogeneous, with a poor correlation between the phenotype and genotype. To date, more than 50 nuclear genes can cause nuclear gene-encoded Leigh syndrome. *NDUFS6* encodes a 13KDa subunit of complex I, which is part of the peripheral arm and is localized in the iron-sulfur fraction of the complex. Mutations in the *NDUFS6* gene were previously reported in only 8 patients from 5 families and all presented with severe neonatal lactic acidemia with complex I deficiency leading to death in the first days of life.

Here, we present a patient with two novel *NDUFS6* mutations to expand the clinical and biochemical spectrum of the disease. Compare to previous reports, this child had a milder phenotype compatible with a Leigh syndrome associated with multiple deficiencies of OXPHOS complexes. Sequencing of a panel of 300 genes involved in mitochondrial disorders allowed the identification of two *NDUFS6* pathogenic variants resulting in almost complete absence of the protein. The first variant c.309+5G>A results in the translation of an unstable protein missing exon 3. The second variant c.343T>C results in a substitution of a cystein located in the Zn-finger domain needed for correct assembly of complex I and a major assembly defect of complex I was revealed by BN-PAGE. These data confirm that the presence of NDUFS6 and the integrity of the Zn-finger domain are essential for correct assembling of complex I.

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E-P06.15

Exome sequencing reveals a novel homozygous mutation in the *RNASEH1* gene in a Polish family with progressive external ophthalmoplegia and ptosis with mitochondrial DNA deletions

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Introduction: Exome sequencing has the power to render molecular diagnostic procedures in an individual patient in the setting of a novel disease, after all standard diagnoses have been exhausted.

Materials and Methods: Whole exome sequencing (WES) was performed for an adult patient with clinically diagnosed mitochondrial encephalopathy with progressive external ophthalmoplegia, ptosis and mitochondrial DNA deletions

The proband was from a consanguineous Polish family with two affected siblings in the pedigree suggesting autosomal recessive inheritance.

Results: Bioinformatics analysis showed a new variant in the *RNASEH1* gene c.493G>C, p.Gly165Arg and predict the damaging potential of the variant.

The *RNASEH1* gene encodes a ribonuclease H1 that specifically degrades the RNA of RNA-DNA hybrids and plays a key role in DNA replication and repair. Mutations in this gene lead to progressive external ophthalmoplegia with mitochondrial DNA deletions (MIM: 616479).

The family studies confirmed biallelic inheritance in two affected siblings. The homozygous variant was not observed in healthy siblings and offspring. The novel variant was not detected in 360 chromosomes from healthy Polish subjects.

Conclusion: This novel variant in the catalytic domain of the *RNASEH1* gene with high probability causes autosomal recessive progressive external ophthalmoplegia with mitochondrial DNA deletions in this Polish family.

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E-P06.16

A novel mutation indentified in *ATP7B* gene by direct sequence of hotspot exons in Moldovan patients with Wilson disease

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Wilson disease(WD) is an autosomal recessive disorder (ATP7B; EC3.6.3.54; OMIM606882) caused by a deficiency of copper transporting P-type ATPase, characterized by accumulation of free-copper(Cu) in liver, brain, kidneys and corneas. The aim of our study was to analyze mutations of hotspot exons of the ATP7B gene in Moldovan patients with WD.

Materials and Methods: We report on 10 WD patients showing unexplained liver disease and/or neurological or

neuropsychiatric disorders, presenting or not of Kayser-Fleischer ring. The tests for serum ceruloplasmin, Cu and free Cu level were used for diagnosis and evaluation of WD. Genetic analysis was performed by "Sanger" genomic DNA sequencing (3500dx Genetic Analyzer, Applied Biosystems Inc.) for hotspot exons 4, 8, 12, 13, 15, 17 and 20 in ATP7B gene.

Results: Diagnosis was based on phenotypic manifestation and laboratory results that reveal decrease of ceruloplasmin level[4,3-24mg/dl] and high free Cu[21-91.1 μg/dl]. Confirmation of diagnosis was based on molecular DNA analysis that revealed a mutation detection rate around 50% of cases, allowing identification of complete genotype in 40%. There was identified 1 novel missense mutation in exone 17 of ATP7B gene (p.A1227T-5%), 2 reported missense mutations (p.H1069Q-30%, p. G1341D-15%) and 1 polymorphism (p.R952K-71,4%). Although p.A1227T mutation was not yet described, different prediction tools were used to reveal its pathogenicity (SIFT-0.001; Polyphen2-0,999) being fortified by biochemical results (ceruloplasmin-4,3mg/dl; free Cu-78μg/dl).

Conclusions: The most common mutation was p. H1069Q in Moldovan WD patients. One novel missense mutation, potential pathogenic, has been found in exone 17 of ATP7B gene(p.A1227T).

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E-P06.17
Identification of a mutation in ABCB4 gene involved in progressive familial intrahepatic cholestasis (PFIC) in bedouin families

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Introduction: Progressive familial Intrahepatic Cholestasis (PFIC) is a group of autosomal recessive disorders characterized by malfunctioning secretion of bile acids or other components of bile. These disorders usually present during infancy or childhood period as progressive conjugated hyperbillirubinemia and liver dysfunction, without proper treatment can lead to death at the first decade of life. PFIC3 is caused by mutations in the ABCB4 or MDR3 gene. This protein is a phospholipid translocator involved in biliary phospholipid excretion. Phospholipids, cholesterol and bile salt form micelles that are essential for the inactivation process of bile salt toxicity and prevent epithelium injury

Study group: Ten members of a Bedouin family with 3 affected children were genetically analyzed. All the patients had cholestasis. Another infant from a different family was also diagnosed based on genetic analysis.

Genetic analysis: DNA was extracted from peripheral blood. Genotyping was done on parents and two affected children using Affymetrix GeneChip Human Mapping 250K Sty arrays. Dedicated software (KinSNP) was used to search the microarray results for homozygous regions consistent with linkage. Homozygosity was detected for chromosome 7 that includes ABCB4, each of the 28 coding exons and their splice junctions of ABCB4 was PCR amplified and sequenced.

Results: A novel mutation c.C433T, causing Gln145Stop was identified in exon 6. It segregated as expected in the family showing homozygosity only in the patients. The truncation of the transporter in the cytoplasmic domain is expected to result in an inactive transporter, thus to be the cause the disease in the family.

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E-P07 Immunology and hematopoietic system

E-P07.01
Compound haplotypes in *CFH* and *CD46* genes, as well as the C3 variants, determine clinical outcome of atypical hemolytic-uremic syndrome- a family-based study

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Atypical hemolytic uremic syndrome (aHUS) is a rare disease consisting of microangiopathic hemolytic anemia,

thrombocytopenia and acute kidney injury, resulting from the alternative complement pathway dysregulation. Clinical presentation and severity of the disease depend on the common (MAF>5%) germline variants in the complement system genes, including haplotypes in the *CD46* and *CFH*, associated with the low ($CD46_{GGAAC}$) or high (*CFH-H3*) risk of unfavorable disease outcome.

Here we describe a family with a clinical follow-up of 11 years. Among 8 siblings, three brothers were affected by aHUS at 22, 27 and 27 yoa, and their niece developed a severe disease at the age of 10 months. We employed next generation-sequencing to analyze the complete sequences of 13 aHUS related genes in all the 11 family members. We also analyzed risk genotypes in 412 unrelated healthy volunteers to verify their population frequency.

The analysis revealed a complex genetic background and familial aggregation of the disease. The 10-month patient was heterozygous for both the MC_{GGAAC} and *CFH-H3* haplotypes, while the adult patients were heterozygous for the *CFH* and homozygous for the *MCP* haplotype, suggesting its protective role. We also identified a potential novel risk genotype in the *C3* gene (AA in rs11569511), present only in the kindred patient, with a population frequency at 1.3%. The analysis also revealed a population frequency of the homozygous *CFH-H3* risk haplotype at 2.6%, underlining the complex pathogenesis of the disease.

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E-P07.03

Candidate Gene Search For Autosomal Dominant Behçet's Disease Through Whole Exome Sequencing

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Normal 0 false false false TR JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin-top:0cm; mso-para-margin-right:0cm; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0cm; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:Calibri; mso-ascii-font-family: Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-ansi-language:TR;}

Introduction: Behçet's disease (BD) is a chronic multi-system inflammatory disorder with uncertain aetiology. inheritance pattern is multifactorial, and most cases are sporadic. A family with four affected members and presenting autosomal dominant inheritance, was studied to search for the putative genetic defect.

Materials and Methods: All affected patients met the International Study Group criteria for BD.. Father and three sibs are affected, and three sibs were unaffected. Exome sequencing was performed in all four affected individuals. Candidate causative variant search in accordance with autosomal dominant inheritance was performed. Heterozygous rare (MAF <0.005), exonic/splicing variants which were present only in affected individuals were prioritized.

Result: Patients did not carry any rare variants in the *TNFAIP3* gene, which is the only known BD related gene. Further, seven rare variants shared by the patients only were chosen as candidates. These are heterozygous c.1756C>T (p.P586S) in *PRG4* (rs757882876), c.35A>T (p.D12V) *in NAGK* (rs150821125), c.1490C>G (p.A497G) in *SLC9A2*, c.3128G>T (p.G1043V) in *PIK3R4* (rs56160735), c.11T>G (p.L4R) in *IL1RAP* (rs200782803), c.379T>C (p.S127P) in *ULBP3* (rs200023001) and c.3581A>G (p.K1194R) in *AHNAK*.

Conclusion: Seven candidate variants in genes involved in immunological and inflammatory pathways were detected. Among the candidates, *IL1RAP* appeared to be the most relevant to the disease. This is the first report suggesting possible *IL1RAP* gene in Mendelian BD. <!--EndFragment-->

S. Ugurlu: None. **G. Turan:** None. **I. Karacan:** None. **S. Dasdemir:** None. **M. Seven:** None. **M.B. Düz:** None. **H. Ozdogan:** None. **A. Tolun:** None. **E. Tahir Turanli:** None.

E-P07.04

Beta thalassemia mutations in patients from Dobrogea, Romania

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Introduction: Beta-thalassemia is a group of hemoglobin diseases caused by a reduction (β^+ thalassemia) or absence (β^0 thalassemia) in the synthesis of beta-globin chains that results in microcytic hypochromic anemia, an abnormal peripheral blood smear with nucleated red blood cells, and reduced amounts of hemoglobin A (HbA) on hemoglobin analysis

Matherial and methods: 26 patients were studied for characterization as either heterozygous or homozygous for beta-thalassemia. Molecular analysis was done by PCR and reverse-hybridization in order to detect 22 of mutations most commonly associated with beta-thalassemia (Mediterranean type).

Results: Of the 22 heterozygous patients 4 (22%) had the β^+IVS1.6[T>C] mutation, 4 (22%) had the β^+IVS2.745 [T>C], 4 patients (22%) had β^0 codon 8[-AA] mutation, 4 patients (22%) had β^0 codon 39[C>T] mutation and 2 (12%) patients had β^+IVS1.110 [G>A]. Three patients were homozygouse for IVS1.6[T>C] mutation and one patient was compound heterozygouse for IVS1.6[T>C] and IVS2.745.

Conclusions: In our study the most frequent mutation was β^+IVS1.6[T>C], contrary to another study who investigate population from Romania and find that β^+IVS1.110[G>A] are the most frequent for our country. This is due because of great ethnic heterogenity of population from Dobrogea. The mutation β^0 codon 8 [-AA] is not identified in the former study.

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E-P07.05
Implementation of a Custom-Designed Targeted Next-Generation Sequencing Panel for the Diagnostics of Inherited Bone Marrow Failure Syndromes

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Inherited bone marrow failure syndromes (IBMFS) composed of great number of clinically similar but genetically heterogeneous diseases with reduction of one or multiple hematopoietic cell lines in bone marrow as a main feature. Next-Generation sequencing (NGS) facilitated the discovery of novel germline mutations that are the cause of IBMFS.The aim of this study was the elaboration and implementation of a custom-designed 173-genes NGS-panel into routine diagnostics. We performed NGS-sequencing for 49 patients with different referral diagnosis: Diamond-Blackfan anemia (17); aplastic anemia (16); Fanconi anemia (3), myelodysplastic syndrome (5); bone marrow failure (3), idiopathic thrombocytopenia (3), dyskeratosis congenita (1), severe congenital neutropenia (1). Sample preparation was carried out with KAPA HTP Library Preparation Kit Illumina (Roche, Switzerland) and tagret hybridization enrichment technique via custom probe panel SeqCap EZ Choice Library (Roche, Switzerland). Obtained libraries were sequenced on MiSeq platform (Illumina, USA).We revealed 27 rare genetic variants that could relate to the cause of the disease (10 - pathogenic, 6 - likely pathogenic and 11 variants of uncertain significance). Among 49 patients the diagnosis was confirmed in 12 and likely confirmed in 7 cases. Thus, total efficiency of revealing causative variants is 38.8%. The use of NGS for diagnostic of IBMFS significantly expands the search range while results can influence the patient's clinical management. Furthermore, identification of IBMFS genetic reason plays important role in genetic counseling of patient's family members. Obtained results show high diagnostic yield and allow to apply this custom gene panel in clinical practice.

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E-P07.06
CBL syndrome as a cause of JMML: new case in a premature neonate with cleft palate, brain anomalies and splenomegaly and review of the literature

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Introduction: CBL syndrome is a rare disease belonging to the group of RASopathies caused by heterozygous mutations of the CBL gene. It has overlapping features to Noonan syndrome such as developmental anomalies, heart defects, bleeding problems, lymphatic dysplasias and hyperextensive joints. Patients with CBL syndrome also have an increased risk to develop juvenile myelomonocytic leukemia (JMML) an aggressive haematopoetic malignancy of early childhood characterized by anemia, thrombocytopenia and hepato-splenomegaly.

Case report: A girl, born at 27 weeks, was referred for genetic evaluation at 3 weeks (corrected age) with cleft palate, brain abnormalities, severe thrombocytopenia and splenomegaly. Dysmorphology assessment highlighted only subtle facial anomalies. 180K CGH-array was normal. Clinical exome sequencing revealed de novo heterozygous point mutation p.(Gln367Pro) in the CBL gene. Because of this result, a bone marrow examination was performed and a diagnosis of JMML was made.

Discussion: Fifty-four cases of CBL syndrome had been described before, four of them had the p.(Gln367Pro) point mutation. The phenotype was quite variable among these patients, usually more subtle than in the NS with heart defects being less common. Cleft palate is not a typical feature and could represent either an expansion of the phenotype or an occasional association. Brain malformations and the development of splenomegaly (and JMML as well) are reported, respectively, in 26% and >70% of patients with the CBL syndrome (including our case), demonstrating how these two features are actually the major ones that allow one to distinguish the CBL syndrome in the RASopathies spectrum.

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E-P07.08
Hemophagocytic lymphohistiocytosis caused by a rare mutation in CTLA4

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Introduction: Hemophagocytic lymphohistiocytosis (HLH) is a rare disorder in children, that is characterized by persistent fever, splenomegaly with cytopenia, hypertriglyceridemia and hypofibrinogenemia. Some of genetic defects (*PRF1, UNC13D, STX11, STXBP2*) in primary HLH have been identified but above 10% have unknown genetic defects.

Materials and Methods: We present two individuals from a previously unreported family identified to have a novel *CTLA4* variant. Proband is a 6-year-old boy, who had a birth weight of 3054 grams and length 56cm. He had normal growth and health until the age of 4. Then, the patient was admitted to the hospital with primary immune thrombocytopenia. The mother of the child was diagnosed with type 1 diabetes and Hashimoto' disease. Physical examination revealed atopic skin inflammation, ulcers of the mucous membrane of the oral cavity and one café-au-lait spot 3x5cm. Immunomodulatory therapy with IvIgG was performed with good but short effect. Chest x-ray showed tumour in mediastinum, CT scan revealed that was fungal. The patient had intensified immunosuppression - initially with cyclosporine and ATG, then started treatment according to the HLH 2004 protocol. Next Generation Sequencing of *CTLA4* detected in a proband and then confirmed in mother a novel, variant *CTLA4* (NM_005214.4): c.356T>C (p.Leu119Pro). The pathogenic variant revealed in a *CTLA4* gene was confirmed by direct Sanger sequencing.

Conclusion: The gene *CLA4* codes a protein being inhibitory receptor acting as major negative regulator of T-cell responses. We expand the mutational spectrum of *CTLA4*-related point out to clinical variability in rare familial cases

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E-P07.09
Case report of primary immunodeficiency in Moldova

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A 4 months and 15 days male infant died in intensive care department, he had bilateral antibiotic resistant pneumonia. Before death he was tested of IgM and IgG for TORCH infections with all negative results. IgM and IgG levels were as low as 0,3 and 2,00 g/l correspondently. Was performed

TREC and KREC concentration measurement through qPCR. KREC level was at 468 copies at 100000 cells, no TREC was detected. According to this data have been assumed diagnosis of primary immunodeficiency (isolated IgM deficiency). Autopsy revealed thymal displasia and hypoplasia with lobulation abnormalities, nodular-glandular reticulo-epithelial stromal reconstruction, Hasall corpuscles agenesis and only unique lymphocytes. It also revealed significant ligament lymphocytic depletion and agenesis of follicular-nodular structures of the organ systems, combined viro-myco-bacterial infection *(CMV +Pn. jirovecii+ A. baumani)* with latent evolution: viral lesions by CMV in generalized form with involvement of lungs, liver, cerebellum, thymus, pancreas, spleen and adrenal glands with development of CMV encephalitis and meningitis, both focal and diffusal necrotico-leucocital pneumocystico-bacterial polysegmented pneumonia with involvement of upper and lower lobes with retractive fibroplastic displazic process in S_1-S_2 of right lobe, erosive-ulcerative leucocytal-necrotic tracheobronchitis. As was mentioned before, tests for TORCH infections were negative, but CMV lesions were detected. It could be due to very low IgM and IgG levels, making their detection impossible. Taking in account patomorphological changes a Nezelof immunodeficiency diagnosis was presupposed. But in difference from Nezelof syndrome, child had low levels of IgM and IgG. This condition makes us doubt if it was especially Nezelof syndrome.

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E-P07.10

KIR genes analysis of four patient's relatives in aploidentical bone marrow transplantation

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Hematopoietic transplantation from human leukocyte antigen (HLA) haplotype-mismatched (haploidentical) donors is an established treatment of patients with high-risk acute leukemia who do not have a matched donor. In haploidentical, T cell-depleted peripheral blood stem cell transplants without posttransplant graft-versus-host disease (GVHD) prophylaxis, donor natural killer (NK) cells play a beneficial role in outcomes. NK cells express, as their only inhibitory receptor for self, a KIR for an HLA-class I group that is absent on allogeneic targets, sense the missing expression of the self-HLA-class I KIR ligand and mediate alloreactions. Donor-vs-recipient NK-cell alloreactivity controls high-risk leukemia relapse. Here we report preliminary data about KIR genes analysis of four patient's relatives in aploidentical bone marrow transplantation to identify the best donor candidate. Donors were analyzed for both the presence/absence of NK alloreactive cells and KIR repertoire characteristics (B content).

Results: Family 1: 2/3 alloreactive, there was no further selection for KIR repertoire; Family2: 3/3 alloreactive, donor selection was based on the KIR repertoire; Family3: donor selection was based on the alloreactivity; Family 4: there was neither alloreactivity nor B content. In this study we purposed to select the best donor candidate in haploidentical, T cell-depleted peripheral blood stem cell transplantations, basing on donor-vs-recipient NK-cell alloreactivity; further more families will be enrolled and will be perform follow up studies monitoring transplantation.

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E-P07.12

NGS of immunoglobulin and T-cell receptor genes repertoire for minimal residual disease diagnostics in pediatric acute lymphoblastic leukemia

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Introduction: Minimal residual disease (MRD) is the major cause of relapse in leukemia and the strongest prognostic factor, allowing to evaluate the efficiency of treatment, and to make a decision on the subsequent therapy. Efficient and sensitive detection of clonal rearrangements of immunoglobulin (Ig) and T-cell receptor (TCR) genes by NGS is applicable in most cases of acute lymphoblastic leukemia (ALL) and used for MRD monitoring along with RT-PCR of fusion transcripts and flow cytometry.

Materials and Methods: Our cohort consisted of pediatric ALL patients aged 4 to 13. Initial detection of patient-specific clonal rearrangements, performed on DNA samples extracted from bone marrow prior to treatment, was carried out by 8 multiplex PCRs of Ig/TCR followed by NGS. MRD detection included targeted NGS of previously detected rearrangements. Quantitative analysis was based on digital PCR-like statistical approach.

Results: We identified over 500 leukemic rearrangements in 87 patients. To evaluate the accuracy of the technique we compared MRD levels of 31 follow-up samples: 16 samples with RT-PCR-measured MRD and 15 samples measured by flow cytometry. In both series both negative and positive results were confirmed by NGS.

Conclusions: MRD detection by NGS is a sensitive and specific method allowing for clonality evaluation and MRD quantification. Given that RT-PCR of fusion genes and flow cytometry in some cases are not applicable for MRD detection, NGS-based detection of Ig rearrangements is likely to become routine procedure for MRD measurement.

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E-P07.13
G6PC3 Deficiency: Mind the Gap between Mild and Severe Neutropenia

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Introduction: G6PC3 deficiency is a recently characterized cause of congenital neutropenia variably associated with multisystem involvement. To date, less than 100 patients have been described. Case report. A 5-year-old girl, born preterm to healthy consanguineous Italian parents, was referred to our hospital for proportionate prenatal-onset growth failure (height and HC -2 SD; weight -4 SD) and mild to moderate neutropenia. Review of records and further investigation highlighted: stomatitis, persistent inflammation of unknown cause (normal faecal calprotectin) but no amyloid deposition, mild global developmental delay, neonatal pulmonary hypertension, congenital heart disease (atrial septal defect, mild tricuspid insufficiency, vascular ring), low serum HDL-cholesterol, renal dysplasia with subnephrotic proteinuria, treated bilateral inguinal hernia, effusive otitis media. Previous findings and appearance on physical examination (prominent superficial veins, triangular facies, unilateral congenital ptosis, epicanthus, bluish sclera, bilateral abnormal palmar creases, proximally placed thumbs, mild clitoromegaly) led to a clinical diagnosis of G6PC3 deficiency. In agreement, combined whole-exome sequencing and autozygosity mapping found the causative genotype NM_138387.3(G6PC3):c.[84_107del]; [84_107del]. At follow-up visits neutrophils ranged approximately from 500 to 1400/μL. Conclusion. The novel homozygous 24-bp indel in exon 1 of G6PC3 we report here is associated with a syndromic phenotype towards the severe end of the clinical spectrum. We also show that G6PC3 deficiency should be considered in the differential diagnosis of congenital neutropenia even when absolute neutrophil count is not below 500.

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E-P07.14
NOVEL VARIANTS IN *ADAMTS13* DETECTED BY NEXT GENERATION SEQUENCING (NGS) IN A PATIENT WITH ATYPICAL THROMBOTIC THROMBOCYTOPENIC PURPURA

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Introduction: NGS is a useful tool to diagnose genetic defects in patients with atypical course of congenital disorders.

Matherials and methods: We report a boy who presented at the age of 7 months with hemolytic anemia, thrombocytopenia and no neurological and renal dysfunction or evidence of thrombosis. He initially responded to steroids and rituximab treatment. Subsequently the relapse of the disease was accompanied by hypogammaglobulinemia. Various primary immunodeficiencies were excluded via Sanger sequencing. Eventually the boy died of intracranial bleeding and sepsis. The clinical exome sequencing (TruSightOne, Illumina) was performed (MiSeq, Illumina) in the patient. Detected variants were filtered, their significance was assessed according to the ACMG recommendations. Candidate variants were confirmed in the patient and his healthy parents by Sanger sequencing, their pathogenicity was tested by *in silico* analysis.

Results: In the patient we detected two novel complex heterozygous variants in *ADAMTS13(NM_139025)* gene*:* c.947_948delinsTT, p.G316V (exon 8) and c.1143_1144delinsC, p.A381fs (exon 10). Each was inherited from respective parent. Both variants have not

been previously reported in the literature and mutations databases. Substitution p.G316V was predicted to be pathogenic by *in silico* analysis.

Conclusion: Familial thrombotic thrombocytopenic purpura(TTP) is a rare autosomal recessive disorder caused by mutation in the *ADAMTS13* gene, encoding the von Willebrand factor-cleaving protease and characterized by microangiopathic hemolytic anemia, thrombocytopenia, neurologic and renal complications due to microthrombi formation. TTP was not suspected in the patient who died before the correct diagnosis was made. Quick access to NGS is crucial in severe congenital disorders.

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E-P07.16
A case report of Wiskott-Aldrich Syndrome with a splicing mutation in WAS gene

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Introduction: Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterized by thrombocytopenia, eczema, and recurrent infections. The incidence of this rare disorder is approximately one to four cases per 1.000.000 live male births.

Material and Methods: We report a six month old boy who had presented with pulmonary septicemia of mixed etiology, secondary thrombocytopenia, eczema and petechial haemorrhagic rash. Immunological investigations have demonstrated essential humoral and cellular changes: low serum level of IgM (0.3mg/L) and IgA (0.2mg/L), elevated IgE (767.6kU/L), low level of CD4(+) T cells (9%) and elevated CD8(+) T cells (59%). Genetic analysis for the detection of a mutation of WAS gene was performed by polymerase chain reaction-single strand conformational polymorphism analysis (PCR-SSCP) and direct sequencing of the PCR product. The primers was taken from Ariga Tadashi et.al., 1997.

Results: In PCR-SSCP analysis, the patient had an abnormal shift band, which was not found in normal controls. In direct sequencing analysis, the patient with WAS showed A-to-G transition at complementary nucleotide 274 (c.274-2 A>G), located in intron 2. A review of family history did not reveal the presence of relatives clinically diagnosed as having WAS and since DNA analysis of the patient's mother revealed no mutation, we can assume that the mutation found in the patient must have occurred spontaneously.

Conclusions: The present study identified a splicing mutation of the WAS gene responsible for WAS in a Moldavian family. To our knowledge, this is the first report on the splicing mutation of WAS gene in Republic of Moldova.

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E-P08 Intellectual Disability

E-P08.01
Different phenotype with 22q13.3 deletion syndrome in two patients

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The 22q13.3 deletion syndrome, also known as Phelan-McDermid syndrome (PHMDS) (OMIM #606232), is a contiguous gene disorder resulting from deletion of the distal long arm of chromosome 22. The deletion occurs near the end of the chromosome at a location designated q13.3. In addition to normal growth and a constellation of minor dysmorphic features, this syndrome is characterized by neurological deficits which include global developmental delay, moderate to severe intellectual impairment, absent or severely delayed speech, and decreased muscle tone (hypotonia). Some people with this condition have autism or autistic-like behavior that affects communication and social interaction, such as poor eye contact, sensitivity to touch, and aggressive behaviors. They may also chew on non-food items such as clothing. Less frequently, people with this condition have seizures.

Patient 1: 9-year-old girl patient Genetic Diseases Diagnosis referred to our center with autism-like behaviors. The chromosome structure was determined as 46,XX. In the constructed arrayCGH analysis, 4315 kb deletion was detected in 22q13.31-q13.33 and 5057 kb duplication was detected in 8p23.3-p22.2 (Agilent 4x180K ISCA CGH +SNP).

Patient 2: A 16-month-old girl was admitted to our Genetic Diagnosis Diagnosis Center with findings of

hypotonia. The chromosome structure was determined as 46,XX. 5205 kb deletion was detected in the arrayCGH analysis 22q13.31-q13.33. In 22q13.3 deletion syndrome, changes in the phenotype of the patient can be seen according to the size of the deletion. Both patients underwent SHANK3 deletion. However, patient 1 included 4315 kb deletions 45 genes, while patient 2 included 5205 kb deletions 63 genes.

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E-P08.02

A case report of a patient with an interstitial deletion in region 2q31.2q32.1

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Introduction: 2q31.2q32.3 deletion syndrome is a rare genetic disorder reported in more than 30 cases up to date. These deletions are responsible for developmental delay, facial dysmorphism, mental retardation and behavioral disturbances. We present a patient with interstitial 2q31.2q32.1 microdeletion and compare his features with those reported previously.

Results: Our patient is a second child of a healthy and unrelated parents. He was born at term, BW 3100 g, BL 50 cm, Apgar scores were 10/10. Cytogenetic prenatal diagnosis due to advanced maternal age was performed and revealed a normal karyotype. During early childhood developmental delay was observed. At first visit, he was 10 years old, with severe intellectual disability, speech developmental delay, mild facial dysmorphism, aggressive behavioral problems worsening with age and occasional episodes of hyperactivity. Array-CGH analysis showed a *de novo* 9563 kb deletion in 2q31.2q32.1 between 179 to 188 Mb. The deletion involves 54 genes, including *CERKL, FRZB, ZNF804A, ITG4A* genes, which, according to literature, may be responsible for specific cognitive and behavioral phenotype and craniofacial abnormalities. We have compered the specific phenotype of our patient with patients diagnosed with 2q31.2q32.3 deletion syndrome reported in literature and we have noticed similarities.

Conclusion: This case of interstitial deletion revealed by array-CGH suggest that 2q31.2q32.1 could be the critical region in 2q31.2q2q32.3 deletion syndrome. The similar reports in the future will be important to definitely define the critical region of this deletion syndrome.

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E-P08.048

p23.3 deletion and 16q23.2-q24.3 duplication detected by arrayCGH in a patient with intellectuel disability

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Balanced chromosome translocations in either parent increases the risk of recurrent miscarriage, unbalanced chromosome rearrangements, congenital malformations, and mental retardation in liveborn offspring. The availability and use of CGH with high-resolution microarrays has greatly improved the detection of micro-deletion and duplications in patients with developmental delay and congenital anomalies. An eleven years old girl referred us with speech delay and intellectual disability. She is the first child of healthy nonconsanguineous parents. She was born at 38th gestation weeks with a weight of 3400 gr. and hospitalized 52 days in the neonatal intensive care unit with sepsis, jaundice and milk allergy. The patient had dysmorphic features including micrognathia, broad forehead, broad nose wings, low-set columella. Cytogenetic analysis was performed and karyotype was 46,XX,der(8)add(8p23). Fluorescent in situ hybridization (FISH) studies were applied in order to confirm the chromosomal region and detected 46,XX.ish dup(16)(q24). High-resolution aCGH +SNP 4x180K platform (Agilent Technologies) revealed 567 kb deletion on chromosome 8 arr[hg19]8p23.3 (191.530,264-770.060)x1 and a 8657 kb duplication on chromosome 16 arr[hg19]16q23.2-q24.3(81.491.655-90.148.393)x3. Parental karyotyping confirmed the chromosomal abnormalities resulted from a paternal balanced translocation involving chromosomes 8 and 16 [46,XY, t(8;16)(p21;q22)]. We described molecular cytogenetic characterization of deletion 8p23.3 and duplication of 16q23.2-q24.3. To our knowledge, this is the first case reported in the literature.

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E-P08.0516

p11.2 CNVs detected by Array-CGH in a cohort of patients with intellectual disability, autism spectrum and obesity

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The presence of a large number of flanking segmental duplications/low-copy repeat sequences with a high degree of sequence identity in the short arm of chromosome 16 (16p) leads to recurrent deletions and duplications as a consequence of non-allelic homologous recombination (NAHR) during meiosis. A recurrent~ 600 kb microdeletion is one of the most frequent genomic imbalances in 16p11.2 (BP4-BP5) associated with abnormal phenotypes including neurodevelopmental disorders, autism spectrum disorder (ASD) and obesity. In a cohort of 26 patients studied by Agilent 180K oligonucleotide array-Comparative Genomic Hybridization (array-CGH) and/or multiplex ligation-dependent probe amplification (MLPA) carrying a 16p11.2 rearrangement (deletion or duplication), 19 (73%) showed a deletion in the classical region of 16p11.2 (29,562-30,192 bp)[hg19]. Although the phenotype of individuals with the deletion can be variable, all patients showed at least one clinical finding typical of 16p11.2 deletion: cognitive impairment, language delay, autism or obesity. Other features less frequent include: neurological issues (epilepsy, neuroimaging findings), behavioural problems, cardiac malformations, vertebral anomalies, macrocephaly, hearing loss. Although the phenotype of 16p11.2 microdeletion syndrome shows a high variability, it represents the second most frequent genetic cause of obesity. The obesity observed in this population may be explained by the haploinsuficiency of one or more of the 30 genes present in this region. On the other hand, it is known that individuals with intellectual disability or autism have a higher predisposition for obesity, possibly due the involvement of one or more pathways.

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E-P08.06

UPF3B gene deletion in a patient with severe intellectual disability, epilepsy, absent speech: problematic of array-CGH coverage

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In the beginning of 2011, we received a 2 year old male patient with severe global developmental delay, microcephaly, epilepsy and short stature, for array-Comparative Genomic Hybridization (array-CGH) analysis. The analysis was performed with Agilent 180K oligonucleotide non-ISCA slide, used in setting at that time, and showed no clinically relevant Copy Number Variant (CNV). At the end of 2017, we were contacted by the referring clinician because a cousin from the maternal side had done array abroad and was diagnosed with a 20Kb deletion at Xq24, involving *UPF3B* gene. The phenotypes of both cousins were very similar. Being a maternal X-chromosome deletion, we revisited the array-CGH result and check if we have missed the deletion. We concluded that *UPF3B* gene was only covered by 2 probes, both with a $\log_2$ ratio compatible with a deletion, and repeated the array-CGH hybridization using an ISCA slide, currently the lab procedure, that contains 36 probes covering the *UPF3B* gene, confirming the deletion, that proved to be maternal. *UPF3B* gene is associated with intellectual disability, autism and facial dysmorphisms, justifying both cousins phenotype. This case illustrates that misdiagnosis can result from technical limitations, namely from inappropriate coverage and highlight the importance of reevaluation of cases previously analyzed by array-CGH and without significant CNVs. The use of arrays with ISCA design is of outmost importance, since they have higher coverage in disease and syndrome associated genome regions, overcoming the general agreement that, at least, 3 consecutive probes are necessary to confidently identify a true CNV.

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E-P08.07

A patient with a new _CNTNAP2_ homozygous variant: further delineation of the CASPR2 deficiency syndrome

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The CASPR2 deficiency syndrome is caused by bi-allelic, loss-of-function variants in the *CNTNAP2* gene and is characterised by intellectual disability and epilepsy onset in the first 3 years of life associated with loss of verbal skills. Exome analysis focused for learning difficulties and epilepsy, identified a novel, homozygous, variant c.985delA, p.(Ser329Valfs*28) in the *CNTNAP2* gene, in a boy with a clinical presentation overlapping Pitt-Hopkins syndrome. This variant has not been previously reported in the literature; however it is predicted to disrupt normal translation of the *CNTNAP2* gene. It segregated from heterozygous, consanguineous, normal parents. The patient was born following a normal pregnancy and delivery. Motor developmental milestones' acquisition was normal but speech was delayed. Generalised, tonic-clonic seizures occurred at 30 months of age and were associated with loss of babbling. At 4 years and 6 months, the patient had a severe, focal epilepsy disorder with varied seizure semiology that responded well to sodium valproate. EEG findings were non-specific and brain MRI showed a slightly smaller, left temporal lobe without cortical dysplasia. The patient had moderate learning difficulties, stereotypic movements (side-to-side, head movements and hand wringing) and episodic panting respiration. We expand the molecular spectrum of the CASPR2 deficiency syndrome and we review the literature to delineate the differential diagnosis of the recessive Pitt-Hopkins syndrome-like disorders.

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E-P08.08

A case of interstitial 6q21-q22.31 deletion with dysmorphic features

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Introduction: Interstitial deletions of the long arm of chromosome 6 are rarely reported. This condition shows phenotypic heterogeneity which probably caused by wide variability in size and localization of the deletion. 6q deletions are classified according to position of the anomaly; proximal (6q11q16), medial(6q15q25) and terminal (6q25qter). To date, the medial 6q deletion was observed in approximately 30 patients. We report a patient with a medial 6q interstitial deletions.

Material and Methods: The proband, a 3-year-old boy, was the second child of healthy non-consanguineous parents. He was referred to our clinic because of dysmorphic features and learning difficulty. His physical examination demonstrated microcephaly, hypertelorism, epicanthus, down-slanting palpebral fissure, frontal bossing, large-simple ear, scoliosis. His weight and height were within normal range.

Results: His cytogenetic evaluation with conventional GTG banding resulted in 46,XY. Considering the phenotypic abnormalities of the patient, array-CGH analysis was performed. 10000 Kb interstitial deletion at 6q21q22.31 bands, arr[hg19] 6q21q22.31(113,754,403-123,754,142)×1, was observed at analysis. The deleted region includes 55 OMIM genes. His parents had normal constitutional karyotype but array analysis not performed yet.

Conclusion: The interstitial deletions of 6q characterized by heterogeneous phenotypes. Intellectual disability, microcephaly, developmental delay and skeletal abnormalities were common among these patients. We compared the phenotype of our patient with those reported in the literature. We hope the clinical features that we present in our case, would be helpful for full comprehension of karyotype/phenotype correlation in patients with 6q deletion.

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E-P08.09

Unravelling the Xp22.3 microdeletion/microduplication in a family with X-linked icthyosis combining aCGH, MLPA and FISH techniques

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Introduction: X-linked ichthyosis is a genetic disorder affecting the skin caused by a deficit in the steroid sulfatase enzyme (STS), and often is associated with a recurrent microdeletion at Xp22.31.

Patients and Methods: The proband is a 17-year-old boy and he was referred to the clinical geneticist because of icthyosis and autism spectrum disorder (ASD). The molecular analysis consisted on Comparative Genomic Hybridization array (aCGH) (Agilent 60K), *Multiplex Ligation Dependent Probe Amplification* (MLPA) self customized to analyze the CNV's inheritance in parents and siblings and Fluorescence *In Situ* Hybridization (FISH) using the BAC clone RP11-359O20 to characterize the CNV.

Results: aCGH analysis of the patient revealed an interstitial deletion on Xp22.3 involving the Xp22.3 region. The deletion encompasses approximately 1.6 Mb (arr[hg19] Xp22.31(6,488,721-8,097,511)x0) and contained *HDHD1, STS, VCX, PNPLA4* and *MIR651* genes. MLPA analysis confirmed the deletion in the proband, reported a duplication in his sister and grand mother and a normal MLPA dosage was shown in his mother and his maternal uncle. The grandfather has icthyosis too but he did not accept genetic testing. In order to decipher the normal MLPA result in the mother we applied FISH that shows a microduplication in one X chromosome and a microdeletion in the other one.

Conclusions: The deletion on Xp22.3 gene is consistent with the principal feature of our patient of X-linked ichthyosis and probably for the ASD. To identification of the mother harbouring both microdeletion and microduplication Xp22.31 found thanks to using FISH allowed to offer a reliable genetic counselling.

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E-P08.11
Novel mosaic CREBBP mutation in a patient with overlapping clinical features of Rubinstein-Taybi syndrome and Floating-Harbor syndrome

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Introduction: We present three-year-old boy with severe developmental and growth delay, agenesis of corpus callosum, toracal scoliosis and craniofacial dysmorphism with prominent forehead, dolichocephalic head, hypoplasia of nose, prominent collumela and smooth philtrum. He underwent metopic craniosynostosis surgery. He does not speak. Based on clinical presentation, Floating-Harbor syndrome (FHS) was suspected. FHS and Rubinstein-Taybi syndrome (RTS) are both rare genetic disorders characterized by multiple congenital anomalies and intellectual deficit. They share clinical features since causative genes belong to the same signaling pathway. FHS is caused by mutations in the *SRCAP* that codes for a protein who activates *CREBBP*. *CREBBP* plays a key role in regulation of cell growth and division and is crucial for normal development. Pathogenic variants in *CREBBP* represent a known cause for RTS.

Materials and Methods: DNA was isolated from peripheral blood lymphocytes using salting out method. Clinical exome sequencing (Illumina TruSight One) which targeted genes related to observed clinical presentation was performed.

Results: Clinical exome sequencing indicated presence of a novel, likely pathogenic, stopgain variant c.6244C>T (NM_004380.2) in *CREBBP*. Variant is present in mosaic state (18,5%).

Conclusion: Presented boy is the first reported patient with the mosaic variant c.6244C>T in *CREBBP*. While mosaic *CREBBP* variants have been described as a cause of clinical presentation with traits associated with Rubinstein-Taybi syndrome, as far as we are aware, a clinical overlap between RTS and FHS has not been previously reported.

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E-P08.12
A clinical case of early-infantile epileptic encephalopathy caused by a de novo pathogenic variant in the STXBP1

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STXBP1-encephalopathy is an early-onset epileptic encephalopathy characterized by different types of intractable seizures, severe intellectual disability, autism-like features, hypotonia. It's caused most frequently by a heterozygous pathogenic variant in STXBP1 or contiguous gene deletion that includes STXBP1 gene which codes an essential protein for presynaptic vesicular release.

We present clinical and genetic characteristics of a 3-years old boy with early-infantile epileptic encephalopathy. The boy was born in term from the pregnancy complicated by gestational diabetes mellitus. Proband's birth weight was 4160 g, length 54 cm, Apgar score 7/8 points. Motor development is delayed – keeps his head from a month, sits from a year, doesn't walk. Speech is absent. From 3 months there is a periodic tremor of extremities. An EEG is

represented by acute waves, polyphase complexes and slow wave activity. Typical epileptiform activity was not detected. A MRI of the brain at 2-years old revealed mild enlargement of subarachnoid spaces.

Phenotype features include upslanting palpebral fissures. The patient has impaired communication and limited eye contact.

Analysis of whole exome sequencing data revealed a previously described pathogenic variant (rs796053361) c.875G>A (p.Arg292Leu) in heterozygous state in STXBP1 in the proband. This variant was not detected in either parent and was confirmed with Sanger sequencing.

Our clinical case describes the phenotype associated with the mutation in STXBP1 and contributes to the evidence of its role of this gene in the pathogenesis of the early-infantile epileptic encephalopathy.

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E-P08.13

Clinical And Genetic Evaluation Of Sotos Syndrome Cases With A Novel Mutation; A Single Center Experience

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Objective: In the literature review we found few studies that included Sotos syndrome findings and clinical manifestations.The aim of this study is to evaluate the prenatal, natal and postnatal clinical findings associated with sotos syndrome.

Methods:16 patients were examined retrospectively in this study, ranging in age between 3 and 23. Parameters that we investigated are; birth weight, birth length,Apgar score at 5 minutes, NSD1 gene, dysmofological face appearance, bone age, seizure, learning disability, feeding difficulties, surgical operation and other accompanying abnormalities (brain MRI, abnormal echocardiographic findigs,have chronic otitis media, etc.).Patients files and genetic reports of patients were examined and the hospital registry system was screened.

Results: İn this study, we found an increase in the frequency of the parameters; breech presentation, APGAR score in the 5th minute is between 4-7, large for gestational age (LGA), atrial septal defect at echocardiography, consanguinity marriage in the Sotos syndrome individuals compared to the normal population.We also found delay in developmental steps compared to the normal population.We determined that all patients had macrocephaly, increased bone age, chronic otitis media frequency and hernia operative frequency consistent with the literature.We identified a new deletion-type mutation in the 19th exon of the NSD1 gene that was not previously reported In the literature. Unlike the cases reported in the literature xyphoidal protrusion was detected at this patient with novel mutation.

Conclusions: Rapid growth, difficulty in learning, macrocephaly, speech delay and skewed personality,it is absolutely necessary to think about Sotos syndrome. Especially timid, shy personality was a bulge available in our all patients.

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E-P08.14

Nonrecurrent 11q12.1q13.3 gain in a patient with motor and cognitive developmental delay, multiple congenital anomalies, and short stature

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Introduction: Neurodevelopmental disorders are characterized by substantial genetic heterogeneity and clinical variability. Hereditary cerebellar ataxias, characterized by clinically variable gait disturbances, are often accompanied by neurological symptoms. The infantile onsets, common in patients with congenital variants, typically have developmental delay.

Material and Methods: Herein, we reported a 10 years old boy who walked at the age of 6, talked at 7, born to non-consanguineous parents. His clinical evaluation showed severe motor, speech and cognitive delays, facial dysmorphisms with epicanthal folds, palpebral fissure, hypertelorism, broad nose, bulbous nasal tip, long philtrum, thin upper lip, high arched palate, irregular teeth, retrognatia, and short stature. Chromosomal Microarray Analysis (CMA) was done using GeneChip® CytoScanHDTM array.

Results: CMA revealed a 12.9 Mb duplication in arr [GRCh37] 11q12.1q13.3(56,928,391-69,867,039)x3, encompassing 408 genes. The genes related to the phenotype were *CLP1, CNTF, STX3, TMEM138, TMEM216, BEST1, UQCC3, ATL3, COX8A, SCYL1, NRXN2, SNX15, SLC25A45, RNASEH2C, AP5B1, PACS1, KLC2, BBS1, SPTBN2, NDUFV1*, and *NDUFS8*. There is evidence that autosomal recessive spinocerebellar ataxia-21

(OMIM616719) is caused by compound heterozygous mutation in the *SCYL1* gene. Furthermore, mutation in *SPTBN2* gene cause spinocerebellar ataxia autosomal recessive 14 (OMIM615386). In both, are observed patients with developmental delay.

Conclusions: The CMA is a powerful tool to investigate children with motor and developmental delays, in order to clinically relevant genetic diagnosis, such as the genetic evidence found for our proband. Therefore, CMA was efficient to help in delineating phenotypic variation and allowed adequate clinical management and better follow up for the proband and his family.

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E-P08.15

Duplication of distal part of 7q11.23 region not encompassing the Williams-Beuren critical region in a patient with moderate intellectual disability and cardiomyopathy

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The hemizygous microdeletion of approximatelly 1.5 Mb region of chromosome 7q11.23, causes the neurodevelopmental disorder known as Williams-Beuren syndrome (WBS). It is a complex multisystemic disorder, with the prevalence of 1:7,500-1:50,000 newborns. Patients with WBS demonstrate various clinical disturbances including cardiovascular manifestations; developmental delay; behavioral and cognitive abnormalities. Microduplication of the 7q11.23 region, known as Sommerville-van-derAA syndrome was also reported, with milder phenotype but also with facial dysmorphism and deficits in social interaction ability. In addition, a dystal 7q11.23 duplication was found as a new entity in 5 cases with mild intelectual disability, agressivnes and authistic spectrum disorders. Influence of the genes *HIP* and *YWHAG* was suggested to be critical for the manifestations of this microduplication syndrome. Here we report a case with a *de novo* 7q11.23 microduplication distal from the Williams-Beuren critical region with a minimal lenght of 1Mb. The case is a five year old boy with cardiomyopathy, but with normal, hypermobile mitral valve and moderate intellectual disability. MRI of the brain indicated widened interhemispheric space. Facial dysmorphism include prominent cheeks, eyelid ptosis, epicanthus, prominent philtrum, small teeth, and everted lower lip. Microduplication was determined using microarray technology, Agilent Sure PrintG3 CGH4x180K oligo micorarray kit, followed by data analysis on Agilent Genomic Workbench Software. Seventeen genes were determined in the duplicated region including *HIP* and *YWHAG* genes. In conclusion here we report a new case with distal 7q11.23 microduplication, with additional information of clinical variability, important for diagnostic purposes, and possibility for adequate genetic counselling for affected families.

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E-P08.17

Threefold imbalance - duplication 6p22.3, duplication 9p24.2, and duplication 13q33.3- in a boy with developmental delay/intellectual disabillity

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Case report: We report on a 9 years old boy with mild intellectual disability, motor delay, severe language delay, and behavioural problems. His body measurements are in the normal range, and he only shows some mild facial dysmorphic signs. His mother and one of his brothers attended special schools.

Materials and Methods: Conventional karyotyping was performed using standard GTG banding technique. For array CGH analysis CytoChip Oligo 180K microarray was used. Segregation analyses were performed by quantitative PCR.

Results: Conventional chromosome analysis showed a normal male karyotype. Array CGH revealed three imbalances: a) A 388 kb duplication 6p22.3 encompassing the genes *NUP153*, *FAM8A1*, *CAP2*, and *KIF13A*. b) A 685 kb duplication 9p24.2 encompassing the gene *RFX3*. c) A 1,71 Mb duplication 13q13.3 encompassing the genes *LIG4*, *TNFSF13B*, *ABDH13*, and *FAM155A*. Segregation analyses proofed the duplication13q33.3 to be maternally inherited, the duplication 9p24.2 to be paternally inherited, and the duplication 6p22.3 to be de novo.

Conclusion: The present case as another good example for the complex genetics of children with developmental delay/intellectual disability and for the problems concerning the classifications of the CNV's in these patients. None of the CNV's in our patient is described in the literature, but for all three some patients are listed in the DECIPHER database. Consequently, all three imbalances were classified as variants of unknown significance (VUS), and presumably only the combined effect is responsible for the patient's full phenotype.

U. Heinrich: None. **M. Cohen:** None. **I. Rost:** None.

E-P08.18

Identification of a novel mutationgt X-linked mental retardation type 102 by whole exome sequencing

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Introduction: X-linked mental retardation broadly refers to a group of inherited disorders characterized by varying degrees of mental retardation. Mental retardation, X-linked 102 is a form of mental retardation characterized by significantly below average general intellectual functioning associated with impairments in adaptive behavior .This study presents a patient with no definite clinical diagnosis. WES (Whole Exome Sequencing) revealed a novel mutation.

Materials &Method: The case is a 7 years old girl with mild mental retardation, delayed motor and speech development, and talipes equinovarus. Brain MRI showed mild dilatation of ventricular system. Karyotype is normal 46XX. Metabolic screening, urine amino acids chromatography, plasma amino acids HPLC, and thyroid function were normal. Age of manifestation was 6 months. Parents are asymptomatic and non-consanguineous. They have no other affected child. WES was requested. Sanger sequencing was used for segregation analysis of the identified variant. Insillico analysis of the newly identified variant was done.

Results: WES revealed a previously unreported heterozygous variant in the DDX3X gene, c.894C>A (p. Cys298*).This is a nonsense substitution which interrupts the reading frame by a premature stop codon.

Conclusion: As this variant was not detected in either parent, we conclude that it is de novo in the index patient. All Insilico analysis supported the pathogenicity of the mutation. This mutation is compatible with X-linked mental retardation type 102. No other phenotypes are known to be associated with pathogenic variants in DDX3X.

M. Javaheri: None.

E-P08.19

A novel *MID2* mutation as a cause of X-linked intelectual disability-101

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A mutation in *MID2* has recently been associated with X-linked intellectual disability-101 (IDX101) in a large kindred with affected males showing variable degrees of intellectual disability (ID), global developmental delay (GDD) and hyperactivity. Other features, present in more than half of the individuals, were speech disorder and strabismus. Dysmorphic features were insignificant, except for a long face.

Herein, we describe two maternal half-brothers with ID/GDD in whom a hemizygous variant, c.668G>A, p.(Arg223His), was identified in *MID2* through trio-based whole exome sequencing (WES), including both boys and their unaffected maternal grandfather. The older brother (14 years old) has severe ID, along with mild autism spectrum disorder (ASD), microcephaly, strabismus, atrial septal defect and dysmorphic features including a long face, downslanting palpebral fissures, bilateral epicantus, long and pear-shaped nose, thin upper lip and retrognathia. The younger brother (6 years old) has moderate GDD with severe language impairment, severe ASD, astigmatism and less marked dysmorphic features (a similar nose and retrognathia). The variant, classified as of uncertain significance (class 3), is absent in the grandfather and present at a very low frequency in population databases and is located in a highly conserved residue of the MID2 protein. Other etiological investigations, including metabolic panel, *FMR1* PCR and array-CGH were normal. Taking all into account, we believe this could be the cause of the boys' phenotypes, being the second family described with IDX101. However, more families need to be reported in order to strengthen this causal relationship.

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E-P08.20

Genetic testing through multiplex ligation dependent probe amplification analysis for children with global developmental delay or intellectual disability

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Introduction: Global developmental delay or intellectual disability (GDD/ID) are relatively frequent clinical conditions for consultation and diagnosis continues to be a challenge when the etiology remains elusive in about 25% to 50% of cases. Identification of microdeletion/microduplication syndromes with DD/ID is currently performed by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis. The aim of this study was to detect the submicroscopic chromosomal aberrations using MLPA in children with GDD/ID.

Materials and Methods: At the Laboratory for Medical Genetics Tirgu Mures, Romania we investigated a total of 56 GDD/ID children with/without congenital anomalies who had normal results of G-banding karyotype analysis, using MLPA kits for common microdeletions/microduplications syndromes and X-chromosome.

Results: The detection rate for submicroscopic chromosomal aberrations was found to be 12.5%. The microdeletions/microduplications detected include regions for DiGeorge syndrome (3 cases), Williams syndrome (1 case), Wolf-Hirschhorn syndrome (1 case), Cri du Chat syndrome (1 case) and Miller-Dieker syndrome (1 case).

Conclusions: Therefore, the MLPA is a cost-effective analysis for identification of submicroscopic chromosomal aberration syndromes, enables a rapid diagnosis of genomic imbalances in patients with GDD/ID with/without congenital anomalies, and the results needed to be confirmed using other types of molecular analysis.

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E-P08.21

Autosomal Recessive Intellectual Disability type 46 caused by homozygous mutation in *NDST1* gene (OMIM#616116): a case report

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Introduction: Intellectual disability comprises a large group of genetically heterogeneous neurodevelopmental disorders with different inheritance patterns. The identification of the underlying genetic cause is a recurrent challenge in clinical genetics. Missense NDST1 mutations have been recently proposed as a cause of autosomal recessive intellectual disability (ARID) with a distinctive phenotype.

Clinical Report: We report on a 6-year-old girl, born to healthy and non-consanguineous parents of Roma origin. Pregnancy and neonatal period were uneventful. Postnatal growth was within the normal range. She presented with marked psychomotor delay, expressive language disorder and hand flapping. She attends a regular school with major support and speech therapy. Her clinical phenotype includes broad nose, large mouth with thick lips, large ears, brachydactyly with tapering fingers, and deep palmar creases.

Results: Brain MRI, renal ultrasound, metabolic analysis, Array-CGH, Fragile-X and Angelman methylation analysis were normal. Angelman-like and BAFopathies targeted exome sequencing yielded normal results. WES revealed a previously reported homozygous missense variant c.1831G>A, p.G611S in the NDST1 gene, that segregated as expected within the family.

Discussion: We report on a new case of ARID type 46 characterized by neurodevelopment delay with impaired expressive language, stereotypies and dysmorphic features. Hitherto, only nine patients from five unrelated families have been published. Due to the association of ID, stereotypies, happy demeanor and macrostomy, Angelman syndrome-like syndromes and BAFopathies should be regarded as differential diagnoses. Our report emphasizes once again the clinical utility of WES in patients with undiagnosed ID.**References:** Reuter M et al, 2014. Najmabadi H et al, 2011.

A. Serrano Antón: None. **V. López González:** None. **M. Sánchez Soler:** None. **M. Ballesta Martínez:** None. **L. Rodríguez Peña:** None. **M. García-Hoyos:** None. **E. Guillén Navarro:** None.

E-P08.22

Bosch-Boonstra-Schaaf optic atrophy syndrome due to NR2F1 gene deletion

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Introduction: Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS, OMIM #615722) is an autosomal dominant disorder characterized by delayed development, moderate intellectual disability, and optic atrophy and/or hypoplasia, described in 2014. Dysmorphic facial features are variable and nonspecific. BBSOAS results from mutations in NR2F1 and findings suggest haploinsufficiency as the pathogenetic mechanism, even though ClinGen

Haploinsufficiency Score for NR2F1 is 0 (being 0 no evidence available and 3 sufficient evidence for dosage pathogenicity), requiring that more information is neccessary to determine the role of NR2F1 haploinsufficiency in BBSOAS.

Material and Methods: We present a 8 years old male patient with delayed speech and language development, visual impairment (bilateral optic tract alteration), poor motor coordination and mild dysmorphic features (left single palmar crease, sacral dimple and left supernumerary nipple). Automatic DNA extraction was performed following blood sample venopuncture (QIAcube, Qiagen) and Comparative Genomic Hybridization array (aCGH) was performed with Agilent SurePrint G3 ISCA V2 CGH 8x60K. Bioinformatic interpretation was done with Agilent Cytogenomics 4 and Cartagenia Bench Lab CNV software. Genome Build GRCh37 and ISCN 2016 recommendations was used to report.

Results: arr[GRCh37] 5q15(92718667_93430350)x1 dn. aCGH showed a 711,7 Kb interstitial heterozygote deletion in 5q15 (chr5:92718667_93430350), encompassing NR2F1 gene. Parental studies determined de novo inheritance.

Conclusions: Bosch-Boonstra-Schaaf optic atrophy syndrome is a recent described disorder. Most of the cases reported are due to mutations in NR2F1 and few whole gene deletions have been described. ClinGen Haploinsufficiency Score for NR2F1 is 0. This finding supports the NR2F1 happloinsufficiency role in the pathogenic mechanism of BBSOAS.

A. Repáraz-Andrade: None. **C. Torreira Banzas:** None. **O. Blanco Barca:** None. **M. Blanco Pérez:** None. **A. Amado Puentes:** None. **C. Melcón Crespo:** None. **M. A. Andrade Olivié:** None. **J.R. Fernández Lorenzo:** None.

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Rare diagnosis of autosomal-recessive Pitt-Hopkins-like syndrome 2 by microarray-analysis

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Introduction: Pitt-Hopkins-like syndrome 2 (PTHLS2) is an autosomal-recessive disorder caused by biallelic mutations in Neurexin 1 (NRXN1). Only a few cases have been published in literature. Characteristic features are severe mental retardation and mild facial dysmorphism. We present a girl with unspecific developmental delay who was diagnosed PTHLS2 by microarray-analysis.

Patient and Method: The patient was seen at the age of 28 months because of global developmental delay. She was able to sit at the age of 12 months, but not able to walk yet. Active vocabulary was limited to less than five words. She had mild unspecific dysmorphism and no major malformations. Microarray-analysis was performed with Affymetrix CytoScan HD SNP-Array. Validation of CNVs and segregation analysis was done by MLPA with self-designed probes.

Results: By microarray-analysis we identified two intragenic deletions in NRXN1 in 2p16.3 that could be confirmed by MLPA. Deletion 1 spans 248 kb (arr [GRCh37] 2p16.3(50373077_50621548)x1) and deletion 2 spans 208 kb (arr[GRCh37] 2p16.3 (51054671_51263065)x1). Segregation analysis revealed compound-heterozygosity as each deletion was inherited from one parent, respectively.

Discussion: To our knowledge this is only the second published case of PTHLS2 with compound-heterozygosity for two intragenic NRXN1 deletions detected by microarray. Harrison et al. described a similar case of two sisters who presented with severe epileptic encephalopathy (Harrison et al., Am J Med Genet 2011). Our case broadens the clinical and molecular spectrum of PTHLS2 but retrospectively also shows that a clinical diagnosis of PTHLS2 would not have been possible as specific symptoms or dysmorphic features are missing.

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E-P08.24

A novel pathogenic variant of *PURA* in a patient with severe developmental delay, delayed myelination and empty sella

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There are several reports that patients with severe neurodevelopmental delay, learning disability, neonatal hypotonia, feeding difficulties, abnormal movements and epilepsy were caused by pathogenic variants in *PURA*. Here we repot a patient with severe developmental delay, neurological and endocrinological abnormalities associated with a novel pathogenic variant in *PURA* identified by whole exome sequencing (WES) analysis. The patient was a 9-year-old boy. The boy was born at 39 weeks of gestation as the first

child of nonconsanguineous healthy parents. His birthweight and Apgar score was 2210 g and 8/9, respectively. The boy showed nystagmus from infancy with delayed myelination in the brain. He was suspected to Pelizaeus-Merzbacger syndrome at 7 months of age, which was excluded. He presented with severe developmental delay, intellectual impairment with no meaningful words, short stature, empty sella and pseudobulbar palsy. Written informed consent was obtained from his parents. WES analysis was performed using Human All Exon V6 kit (Agilent) and Hiseq 2500 (Illumina). The detected variants were confirmed using Sanger sequencing. A novel heterozygous 8-bp insertion in the *PURA* gene was found in the patient. The variant was *de novo* and it causes frameshift and premature stop codon. The variant is at the N-terminal PUR repeat region I related to severe developmental delay. *PURA* is one of the primary responsible genes for 5q31.3 microdeletion syndrome. Thirty-two patients with neurodevelopmental disorder and brain abnormality caused by *de novo* variants in *PURA* have been reported. Our finding and those reports suggest that loss-of-function of PURA causes the disease.

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E-P08.26
PURA syndrome: a case study

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Background: PURA syndrome is a recently described neurodevelopmental disorder characterized by moderate-to-severe intellectual disability, epilepsy, feeding difficulties, hypersomnolence and hypotonia. To date, genotype-phenotype correlation has been observed to have no significant differences between mutation classes and disease severity.

Case presentation: We report a 15 years old Chinese female, who is the only child of non-consanguineous parents. Antenatal and birth history were unremarkable. As a neonate, she required tube feeding for somnolence and poor suck and also had apnea and desaturation episodes without need for intubation. She had global developmental delay. She walked independently at 3 years old. She had seizure-like movements at 5 years old with a normal EEG. However, she developed tonic seizures with an abnormal EEG at 12 years old, in which sodium valproate had been advised. When she was first seen in the Genetics clinic at 12 years 10 months old, she remained non-verbal with no stereotypic hand movements and had regression in gross motor skills since the onset of seizures. She has short stature (-2.8 SD), moderate scoliosis, myopathic facies and no obvious facial dysmorphism.

FISH for Prader-Willi syndrome, chromosomal microarray analysis (CMA) and urine glycosaminoglycans (GAGs) were normal. Whole exome sequencing (WES) performed subsequently detected a novel, de novo heterozygous c.154dupG (p.L54fs*) variant in *PURA* gene, consistent with the diagnosis.

Conclusion: Even though a novel variant has been detected, our patient's phenotype is consistent with the core features of PURA syndrome. Further studies are needed to better understand genotype-phenotype correlation associated with PURA syndrome.

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E-P08.27
A large deletion of the *MECP2* gene in a Japanese patient with severe phenotype of Rett syndrome

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Methyl-CpG- binding protein 2 (MECP2)-related disorders in females include typical Rett syndrome (RTT, OMIM#312750), variant RTT, and mild learning disabilities, which are inherited in an X-linked manner. Typical RTT is recognizable by arrested development between 6 and 18 months of age, and characterized by regression of acquired skills, loss of speech, stereotypic movements and mental retardation. Mutations in *MECP2* are identifiable in 80〜90% of individuals with typical Rett syndrome but less frequently in atypical RTT.

Clinical Report: A patient is 7-year-old girl born to non-consanguineous parents at 40 weeks with birth weight of 2484g (-2.1 SD), height of 47 cm (-1.4 SD) and head circumstance of 31.5 cm. (-1.5 SD). Amniotic fluid karyotyping showed 46,XX. She has severe neurodevelopmental disorder characterized by psychomotor regression with the development of distinctive hand stereotypies, seizure, breathing abnormalities, and gait apraxia. Her thrive has decreased; current weight, height and head circumstance are 15.5 kg (-1.9 SD), 105.5 cm (-2.6 SD), 47.7 cm (-2.4 SD), respectively. Although she was clinically suspected RTT, no pathogenic variant was detected in *MECP2* by direct-sequencing method.

Results and conclusion: Whole exome sequencing (WES) analysis suggested that there was a heterozygous large deletion between the exon 4 of *MECP2* and the upstream region of *IRAK*. Long-range PCR and sequencing revealed that the deletion was spanning about 9 kb at 3' region of the *MECP2* gene. The large deletion might affect severe phenotype of RTT due to loss of terminal codon and/or the polyadenylation sites.

K. Yanagi: None. **M. Minatogawa:** None. **M. Iso:** None. **K. Satou:** None. **N. Okamoto:** None. **Y. Matsubara:** None. **T. Kaname:** None.

E-P08.28

Marfanoid habitus in two patients with a mutation in the SATB2-gene

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Background: *SATB2*-associated syndrome (SAS) is a multisystem disorder characterized by developmental delay / intellectual disability with limited to absent speech, behavioral issues and craniofacial anomalies. Less common features include several skeletal anomalies (e.g. pectus deformities and scoliosis). While dysmorphic features have been described in individuals with this condition, these features are not described as typically distinctive enough to allow for a clinical diagnosis of SAS.

Patients: We describe two patients with intellectual disability and a de novo SATB2-mutation. They both show a remarkable marfanoid habitus, including tall height, slender build, arm span > height, arachnodactyly, high arched or cleft palate.

Conclusions: These findings might highlight a recognizable marfanoid phenotype in at least part of the patients with *SATB2*-associated syndrome.

M. Sinnema: None. **J. Nicolai:** None. **R. Dash-Vorstenbosch:** None. **S. Stegmann:** None.

E-P08.29

TRAPPC9 missense variant may associate with dysmyelination

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Introduction: A 17-year-old female patient was referred to us with complaints of severe intellectual disability, psychomotor retardation, epilepsy, and operated cataract. Parents were first cousins.

Physical examination: Spelling a few words, short stature, obesity, hypotonia, mild spasticity of lower extremities, friendly attitude, forward-leaning posture, and ataxic gait.

Facial features: Bitemporal narrowing, hypoplastic suborbital ridges, strabismus, bilateral epicanthus, bullous nasal tip, strabismus, downturned mouth, deep filtrum, and maxillar hypoplasia. Brain MRI showed bilateral dysmyelinating areas adjacent to the posterior horn of lateral ventricles. MR spectroscopy or routine metabolic screening detected no abnormality.

EEG: Focal paroxysmal activity in right parietooccipital location.

Materials and Methods: Sequence analysis was performed on DNA from the peripheral blood sample using TruSight One sequencing panel (4813 genes).

Results: Sequence analysis revealed c.434G>A variant causing a non-conservative Arg145Gln alteration in amino acid sequence, classified as Variant of Unknown Significance (VUS). ClinVar harbors single missense variant with likely pathogenic effect (GeneDx, clinical testing). phyloP and phastCons estimated pretty high scores (3.167 and 0.997, respectively), either SIFT and PolyPhen predicted this alteration as tolerated.

Conclusions: Loss-of-function variants in TRAPPC9 are associated with "Mental retardation, autosomal recessive 13". ACMG classification lets us determine c.434G>A as VUS, but the similar facial gestalt of the proband with a previous case reported by Marangi et al. (2013) persuaded us to deduce this variant as likely pathogenic. c.434G>A might be causal for the neurological outcome and facial features. Functional validation or statistical evidence is needed to prove the pathogenicity.

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E-P08.30

***TRIO* gene deletion due to pericentric inversion on chromosome 5chromosome 5**

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We present a case study of a 5 year old boy using cytogenetic and molecular cytogenetic approaches presenting with microcephaly, mild intellectual disability and global developmental delay. He is the first child born to non-consanguineous healthy parents. Following an uncomplicated pregnancy, the newborn was delivered at 41 weeks with normal head circumference of 36.5 cm. There was perinatal acidosis. First words were spoken around 15 months of life, walking without help was at the age of 18 months, and he has been clean since the age of three. IQ value at four years was about 73. Other observed features were epicanthus of eyelids on both sides, discrete strabismus, short fingers, and a shawl scrotum.Fragile X syndrome was ruled out at the age of four years. Cytogenetic analysis revealed a male karyotype with a pericentric inversion of chromosome 5 [46,XY,inv(5)(p15.2q31.1) mat]. Microarray CGH analysis was initiated to further characterize the rearrangement and check for imbalances at the inversion breakpoints. Two microdeletions were detected: in 5p15.2p15.1 spanning 2.51 Mb encompassing five OMIM genes, and in 5q.23.1 spanning 1.9Mb, three OMIM genes, respectively [arr[hg19] 5p15.2p15.1(13,017,195-15,528,016)x1, 5q23.1(11,689,3086-118,788,712)x1]. In the 5p15.2p15.1 region is amongst others the *TRIO* (MIM 601893) gene. An association between heterozygousloss of function mutations in the *TRIO* gene and mild intellectual disability with microcephaly was reported (autosomal dominant mental retardation-44 – MRD44, MIM 617061). Karyotyping of both parents revealed that the mother carried an identical inversion on chromosome 5 as well as both microdeletions as shown by array CGH.

N. Dragicevic: None.

E-P08.31

A clinical case of autosomal dominant mental retardation type 49

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Introduction: Autosomal dominant mental retardation type 49 was first described in February 2017. At the moment 17 patients with this syndrome and mutation in the TRIP12 gene are described. The patients have a highly variable phenotype.

Material and Methods: Whole exome library was prepared from genomic DNA using TruSeq DNA Exome (Illumina) and sequenced on a NextSeq550 (Illumina). One pathogenic variant was validated in the proband and her parents by Sanger sequencing.

Results: We present clinical and genetic characteristics of female patient 4-year old undifferentiated mental retardation. The girl was born from 4th pregnancy, delivered physiologically. Proband's birth weight was 2750 g, length 50 cm, Apgar score 7/8 points. Motor development is delayed – keeps her head from a 4 month, sits from 10 month, walks from 1,5 year. Speech is absent. Neatness skills are not completely formed, self-service – are gradually being formed. By 20-minutes EEG a typical epileptiform activity was not detected, but neurophysiological immaturity, signs of dysfunction of cortical-subcortical structures were revealed. Ultrasound of the head revealed abnormalities of the differentiation of the cortex structures and myelin.

Objectively phenotype: motor awkwardness, not pronounced dysmorphic facial features including deep set eyes, macrostomy, long filter, ear lobules. Focal neurological symptoms were not revealed. Exome sequencing revealed de novo heterozygous variant in *TRIP12* (c.3759_3760del, NM_004238).

Conclusions: The patient with the rare autosomal dominant mental retardation type 49 was described to have a novel mutation c.3759_3760del in *TRIP12* gene.

O. Levchenko: None. **E. Dadali:** None. **A. Lavrov:** None.

E-P08.32

X-linked syndromic mental retardation, Nascimento – type identified by Whole Exome Sequencing

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Introduction: X-linked intellectual disability (ID) type Nascimento (MIM #300860), also known as ubiquitin-conjugating enzyme E2 A (UBE2A) deficiency syndrome, is characterized by moderate to severe intellectual disability (ID), speech impairment, dysmorphic facial features, genital and skin abnormalities. UBE2A gene (Xq24) has a role in DNA repair, fertility, and memory formation. All patients carrying UBE2A mutations are males with mothers with normal IQ.

Materials and Methods: A 14-years old male patient with moderate mental retardation,obsessive-compulsive disorder and morphological features such as myxedematous appearance, almond-shaped eyes, hypertelorism, short neck, hirsutism, brachydactyly,abnormal dermatoglyphic pattern and small penis presented for clinical evaluation.Severe atopic dermatitis was obvious.As an infant he had

hypotonia and large fontanels. Standard karyotype was normal, 46, XY and molecular analysis for Prader-Willi syndrome was negative. We applied Whole Exome Sequencing (WES) which analyses 214.405 exons dispersed throughout the genome.

Results: A hemizygous pathogenic deletion of 14 nucleotides UBE2A:c.421_434del14 was indentified in exon 6 of the UBE2A gene by WES analysis. The variant has been confirmed by Sanger sequencing. This deletion causes a frameshift predicted to result in a premature stop codon . This variant was novel but due to its truncating nature it is classified as a pathogenic. Parental DNA analysis of the same variant proved the de novo origin.

Conclusion: We identified a novel mutation associated with X-linked syndromic mental retardation, Nascimento-type. The use of NGS technologies help to establish the diagnosis in patients with mental retardation and an atypical phenotype, who until recently remained undiagnosed.

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E-P08.33

Novel *WAC* frameshift variant in a boy with DeSanto-Shinawi syndrome revealed using whole exome sequencing

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DeSanto-Shinawi syndrome is a rare autosomal dominant neurodevelopmental disorder caused by mutations in the *WAC* gene. It is characterized by global developmental delay, hypotonia, behavioral, sleep and feeding problems, eye abnormalities, constipation and seizures. Facial features can be mildly dysmorphic but are nonspecific. In total, 18 patients have been reported. We describe an 18-year-old boy who has been examined since preschool age because of hypotonia, speech disorder and strabism. His facial phenotype was not remarkable, but with advancing age features such as broad, prominent forehead, bushy eyebrows, deep-set eyes, depressed nasal bridge, bulbous nasal tip, low-set posteriorly rotated ears, wide mouth with thin upper lip and broad chin became more pronounced. He had abnormalities of extremities, abnormal CNS MRI findings and recurrent respiratory infections. He suffered from underweight affecting fat and muscular components, and showed anxiety and autistic traits. Previous specialized investigations failed to explain the etiology of his affection. Exome sequencing revealed a *de novo* heterozygous *WAC* frameshift variant NM_016628.4:c.383del, p.(Pro128Leufs*64) which was absent from all databases. All other patients with *WAC* mutations also carried truncating variants spread across the gene. The long follow-up of our patient allowed delineating of the *WAC*-related phenotype and its developmental trajectory. The facial phenotype was consistent with previous cases, but we show that the main features become more pronounced with age. Nevertheless, the guidance from phenotype is rather limited and it is likely that additional cases of this syndrome will continue to be identified via the genotype-first approach. Supported by 17-29423A and 00064203.

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E-P08.34

Syndromic intellectual disability and developmental delay caused by novel *de novo* truncating variant in AHDC1 gene

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Introduction: Xia-Gibbs syndrome is a rare genetic disorder with autosomal dominant inheritance caused by heterozygous mutations in AHDC1 gene. This condition is characterized by neurological manifestations that include psychomotor delayed, intellectual disability and corpus callosum hypoplasia with distinct facial features.

Case report: We present a 13 years-old female from Colombia, born to non-consanguineous parents. She was diagnosed at age of 2 years for psychomotor and language delay, facial dysmorphic features and sleep apnea with plagiocephaly. She has associated behavioral disorders that include self-harm, poor social interaction with isolation.

Results: Chromosome analysis was normal (Kariotyping and CGH-array). WES (Whole Exome Sequencing) was performed at 12 years and revealed a novel heterozygous *de novo* frameshift variant c.1529delG (p.Gly510Alafs*12) in *AHDC1* gene (NM_001029882.3), variant functional prediction software tools Mutation tester, Polyphen-2, and SIFT classified it as a deleterious variant.

Discussion: The mutation reported here introduces a stop codon at the amino acid 522 of AHDC1 protein (1603 amino acids). This leads to the loss of one DNA-binding motif and PDZ carboxyl-terminal domain, which could

truncate its interaction with other proteins and can be related to the neurobehavioural manifestations in our patient.

Conclusion: The genotype-phenotype correlation in patients with Xia-Gibbs syndrome is not understood. The patient reported by us is the second case in Colombia and differ from previously reported in literature for absence of corpus callosum hypoplasia described in 40% of cases and for her severe neurobehavioral disorder that could be being modulated for the novel frameshift mutation that truncated protein early.

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E-P08.35

Xp11.22 microduplication including IQSEC2 gene in a male with intellectual disability, epilepsy and dysmorphic features

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X-linked intellectual disability (XLID) is a group of genetically highly heterogeneous disorders and one of the most frequent genetic causes of ID occurring in 5-10% of all affected male individuals. Around 100 genes have been considered as determinant of XLID and the role for many of them remains to be elucidated. Here, we described a male with ID, epilepsy, severely impaired speech, abnormal behavior with hyperactivity and some aggressiveness, optic nerve atrophy and craniofacial dysmorphisms as brachycephaly, frontal hair upsweep, prominent nose and ears, everted lower lip, narrow palate, dental crowding, among others; hands and feet minor anomalies were also observed. Array-CGH (Agilent human genome G3 SurePrint 8x60K microarray) disclosed a 416 kb duplication which extends from ChrX (hg19): 53,253,932 to 53,670,215. This variation was confirmed by MLPA technique and Real Time PCR analysis showed the maternal inheritance. The duplicated region encompasses the *IQ motif-and Sec7 domain-containing protein 2 gene* (*IQSEC2*), which has a significant role in the brain maintenance and homeostasis. Although a consistent phenotype of non-syndromic XLID was observed in individuals with *IQSEC2* alterations, the additional features observed in present patient suggest a syndromic form related to Xp11.22 duplications and also support the hypothesis that *IQSEC2* has a role in pathogenesis of syndromic XLID. In addition, the inclusion of *IQSEC2* variations among the causal factors when evaluating ID patients with seizures could be considered.

Clinical details of male patients with Xp11.22 submicroscopic duplications involving the IQSEC2 gene

Patient [Reference]	Intellectual Disability	Epilepsy	Language development	Visual impairment	Behavioural disturbances	Other clinical features	ChrX(hg19) Coordinates and Duplication size
Present Case	Mild-Moderate	Neonatal seizures	Severely impaired speech	Optic nerve atrophy, enophtalmia	Hyperactivity, aggressive behavior	Corpus callosum agenesis and slight ventricular system enlargement, craniofacial dysmorphic features, including enophtalmia	53,253,932-53,670,215 416 kb
A009 [Froyen et al., 2008]	Mild	–	Speech delay	–	Hyperactivity	Normal facial features	53,220,275-53,981,275 761 kb
A057 [Froyen et al., 2008]	Mild	–	Limited speech in later life	–	Hyperactivity	Not present	52,987,689-53,712,958 725 kb
A119 [Froyen et al., 2008]	Mild-Moderate	Febrile seizures	Speech delay	–	Attention deficit hyperactivity disorder	No significant dysmorphic features	52,825,617-53,662,768 837 kb
AU88848 [Froyen et al., 2012]	Mild	–	–	–	–	–	53,169,907-54,101,252 931 kb
FTD[Froyen et al., 2012]	Mild	Cortico-subcortical dysfunction	Speech delay	–	Attention deficit hyperactivity disorder	Functional heart murmur, chronic vomiting and diarrhea, urolithiasis, bilateral inguinal hernia, cryptorchidism, facial dysmorphic features	53,198,95-54,237,527 1,038 kb
F538 [Froyen et al., 2012]	Moderate	–	Limited speech, Stutter	Unequal pupils	–	Large head circumference	53,216,303-54,239,670 1,023 kb
ON1[Froyen et al., 2012]	Mild-Moderate	–	Partial lack of speech	Microphthalmos	Hyperactivity and attention problems, self-destructing behavior	Facial dysmorphic features	52,982,784-53,721,295 730 kb
Patient 1 [Tran Mau-Them et al., 2014]	Severe	Generalised myoclonic seizures	Not aquired	Hypermetropia, strabismus	Stereotypic hand movements	Neonatal hypotonia, postnatal microcephaly, hyperkinesia, normal facial features	53,283,513-53,325,284 42 kb
Patient 3 [Tran Mau-Them et al., 2014]	Severe	Partial epilepsy	Regression	Strabismus	Midline stereotypic hand movements	Neonatal hypotonia, cerebral atrophy, nonspecific hypersignal foci in periventricular white matter, minor facial features	53,276,030-53,298,472 22 kb
P611 [Santos-Rebouças et al., 2015]	Moderate	Not present	Speech delay	Enophtalmia	Hyperactivity and attention problems, aggressive behavior	Abnormal gait, brachycephaly, enophtalmia, dysmorphic facial features	53,316,256-54,074,258 758 kb
P3272 [Santos-Rebouças et al., 2015]	Moderate	Seizures	Speech delay	–	Hyperactivity	Dysmorphic facial features	53,228,169-54,133,735 905 kb
Patient 1 [Moey et al., 2016]	Not determined	–	Speech delay	–	Poor socialization, behavioral problems	Downward corners of the mouth	52,954,520-53,315,542 361 kb
Patient 2 [Moey et al., 2016]	Mild-Moderate	–	–	–	Autism spectrum disorder, challenging behavior, physical aggression, avoided eye contact	–	52,911,287-53,315,010 403 kb
Patient 3 [Moey et al., 2016]	Global delay	–	Speech delay, poor pronunciation	–	Significant behavioral difficulties requiring a special education class	–	52,789,239-53,368,927 579 kb
Patient 4 [Moey et al., 2016]	Severe	Constant generalized sharp slow discharges	No words, little receptive language	–	Smile and shaking hands	Hypotonia, microcephaly, hypogonadism, myoclonus, not walk, dysmorhic facial features	52,341,517-53,782,896 1,441kb

P.P. Henrique: None. **C.R. Lincoln-de-Carvalho:** None. **M.P. de Mello:** None. **A.P. Marques-de-Faria:** None.

E-P08.36

New case of a *de novo* mutation at *ZYMND11* gene resembling the 10p15.3 microdeletion syndrome

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Introduction: In 2012, a new microdeletion syndrome affecting the short arm of chromosome 10 (10p15.3) was described. The implication of *ZMYND11*, a gene located within this region, in the phenotype (essentially characterized by developmental and motor delay, craniofacial dysmorphism and hypotonia) was also hypothesized.

Patient and methods: We present the case of a 8 month male referred to our consultation due to developmental delay and facial dysmorphisms. He was the second child of a non-consanguineous couple born after an uneventful pregnancy with a normal perinatal period. His elder brother presents autism without specific phenotypical features.

At 8 months, he presented hypotonia, growth failure, bilateral cryptorchidism and hypospadias.

Neurophysiological studies and biochemical analyses showed normal results.

Nowadays (6y), as major features, he has no verbal language and presents a peculiar facial phenotype with hypertelorism and narrow palpebral fissures, low-set ears, prominent crus and auricular pit, among others.

Informed consent was obtained from his parents for subsequent studies: conventional karyotype, aCGH, Prader Willi Syndrome-associated defects and exome variants were studied.

Results: Karyotype and aCGH analysis revealed no alterations. Prader-Willi Syndrome was discarded (neither methylation nor CNV alterations were found at 15q11 region).

Exome analysis revealed the presence of a previously described pathogenic variant on *ZMYND11* gene [c.1798T>C (p.Arg600Trp)]. Family analysis (parents and brother) confirmed its *de novo* origin.

Conclusion: The clinical and molecular findings in our patient confirm the association of this identified variant with the described phenotype and the implication of *ZMYND11* gene in 10p15.3 deletion syndrome.

M. García-Barcina: None. **M.A. Fernández-Cuesta:** None. **B. Barreña:** None. **E. Santamaría:** None. **C. Ruiz-Espinoza:** None. **M.I. Tejada:** None. **N. Ibarluzea:** None. **G. Pérez de Nanclares:** None. **E. Sarasola:** None.

E-P08.37

A recurrent de novo mutation and a second variant of unknown significance in *ZSWIM6* in a boy with severe intellectual disability, microcephaly, strabism and hyperopia

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Recently, a recurrent de novo nonsense variant (c.2737C>T [p.Arg913Ter]) in the penultimate exon of *ZSWIM6* was reported to cause intellectual disability and additional central and peripheral nervous system symptoms by Palmer et al. (Am J Hum Genet. 2017 Dec 7;101(6):995-1005), but not frontonasal or limb malformations, which was the phenotype initially discovered with a recurrent missense mutation (c.3487C>T [p.Arg1163Trp]) by Smith et al. (Am J Hum Genet. 2014 Aug 7;95(2):235-40.). We present the phenotype and genotype of a boy with severe intellectual disability, strabism, hyperopia, microcephaly and undescended testis. The molecular karyotype was normal. Exome analysis revealed the recurrent *de novo* nonsense variant (c.2737C>T [p.Arg913Ter]) in the penultimate exon of *ZSWIM6* and in addition a heterozygous missense variant (c.3119G>A [p.Arg1040His]) that was predicted by several tools to be probably pathogenic. This variant is present in dbSNP (rs192222164) and in gnomAD (f= 0.0003779) and was inherited from the unaffected mother. The phenotype of our patient as evident until now fits into the spectrum described with the recurrent de novo nonsense variant. It remains to be elucidated whether the maternally inherited missense variant might be relevant in a possible recessive mode of inheritance. Anyway, the missense mutation of our patient complicated the interpretation of the mode of inheritance in our patient. Our findings widen the spectrum of genotypes and phenotypes associated with *ZSWIM6*.

I. Bader: None. **A. Gamper:** None. **M. Preisel:** None. **W. Sperl:** None. **J. Mayr:** None.

E-P09 Neurogenetic and psychiatric disorders

E-P09.01

A microdeletion including the gene *NUS1* demonstrating interpretation problems, expansion of the phenotype and treatment modification

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Chromosomal microarray (CMA) is nowadays recognized as essential in the evaluation of autism and developmental delay. CMA interpretation depends on databases as ClinGen, DECIPHER and others, which lack information, and original papers are still essential in deciphering the significance of microdeletions/microduplications. We present an 8.5 years old boy, with intractable epilepsy, autistic spectrum disorder (ASD), hypotonia during infancy, strabismus and behavioral problems. Multiple EEG examinations revealed frequent generalized interictal and ictal slow spike & wave discharges. He has various seizure types, and is treated with a combination of valproic acid, clobazam and ethusuximide with a relative improvement but partial control. The child exhibited behavioral problems including aggression and violence, with recurrent rage attacks. Levetiractam administration escalated the frequency of these behaviors significantly, and therefore was discontinued. Metabolic workup was normal including: lactic, ammonia, blood amino acids, Carnitine, Acylcarnitine. A chromosomal microarray performed demonstrated a deletion on chromosome 6: arr[hg19]6q22.1(117,431,000-118,040,939)x1. This 609kb deletion including the *NUS1* gene is located within the minimal essential critical region on chromosome 6q22. The region is known for autism, seizures, tremor and mild dysmorphic features, but less known for behavioural problems as in the case presented. The databases mentioned had only scarce information on the microdeletion, and we found only one paper interpreting it. This work further delineates this rare genetic disorder. We suggest that behavioral problems are part of this microdeletion, and suggest caution using Levetiractam in these patients. We emphasize the importance of early genetic evaluation in children with ASD and epilepsy.

E. Banne: None. **S. Josefsberg:** None. **J. Rosensaft:** None. **H. Bassan:** None.

E-P09.02

Adrenoleukodystrophy for the first time in Bulgaria: two genetically verified cases, one novel mutation

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Adrenoleukodystrophy (MIM# 300100) represents an X-linked recessive disorder and results in accumulation of saturated very long fatty acids in tissues throughout the body. The manifestations of the disorder occur primarily in the adrenal cortex, the myelin of the central nervous system, and the Leydig cells of the testes. Adrenoleukodystrophy is caused by mutations in the *ABCD1* gene (MIM* 300371) located on the Xq28. *ABCD1* codes a peroxisomal membrane transporter protein (ALDP), member of the ATP-binding cassette (ABC) transporter superfamily. Here we report three patients with clinical diagnosis of X-linked adrenoleukodystrophy were referred for *ABCD1* analysis. In two of them Sanger sequencing showed the presence of a molecular genetic variant in the *ABCD1* gene thereby confirming the clinical diagnosis. In the first patient an already reported missense mutation was found - c.1552C>T, p.Arg518Trp, and in the second - a novel still unpublished variant c.2002A>G, p.Thr668Ala. Multiple prediction software tools (PolyPhen-2; MutationTaster; SIFT) define p.Thr668Ala as deleterious. Furthermore, it was not recorded in the population genetic variation database dbSNP v138 and was not found in 60 000 control subjects in the ExAC project affirming the probable disease causing character of p.Thr668Ala. Unfortunately, the third patient did not show any pathological variations in the *ABCD1* gene supposing the need for diagnosis revision. To the best of our knowledge the present study comprises all clinically and genetically diagnosed adrenoleukodystrophy patients in Bulgaria and enriches the spectrum of reported *ABCD1* mutations.

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E-P09.04

Cholesterol related gene polymorphisms in Alzheimer Disease

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Introduction: Numerous genetic evidences pointed out that variations in cholesterol related genes may be associated with Alzheimer Disease (AD) risk. We aimed to investigate the association between polymorphisms in cholesterol related genes and AD in a cohort of Turkish patients. Therefore we have selected *APOA5*_(rs662799), *APOC1*_(rs11568822), *APOD*_(rs1568565), *CH25H*_(rs13500), *LDLR*_(rs5930) and SORL1_(rs2282649) gene polymorphisms that have been previously showed significant association with AD risk.

Materials-Methods: The study group consisted of 257 AD patients (mean age: 75.9 ± 10.4) and 414 healthy controls (mean age: 62.2 ± 13.1). Genotyping was performed by real-time polymerase chain reaction using hydrolysis probes in Light Cycler 480. Genotypes between groups were compared by Pearson x2 test and multivariate regression analysis was performed to analyse the accumulation effect of *APOE* ε4 allele.

Results: Our results showed that the "TT" genotype of *CH25H*_rs13500 polymorphism was significantly more frequent in AD group ($p = <0.001$) and individuals carrying *CH25H* "T" allele per se, had increased risk for AD (OR=3.27, 95%CI=2.0-5.35, $p = <0.001$). The "ins/ins" genotype of *APOC1*_rs11568822 was significantly more frequent in AD group compared to controls ($p = <0.001$). However no significant association with AD risk was found in *APOC1* insertion allele carriers that did not harbor *APOE* ε4 allele.

Conclusions: Our results suggest that *CH25H*_rs13500 polymorphism is associated with AD risk in the Turkish population and *CH25H* might have a role in pathogenesis of AD independent of *APOE*. Association between *APOC*1 "ins" allele and AD risk can be explained by linkage disequilibrium with the *APOE* locus.

G. Guven: None. **E. Vurgun:** None. **B. Bilgic:** None. **H. Hanagasi:** None. **H. Gurvit:** None. **E. Lohmann:** None. **E. Ozer:** None. **N. Erginel-Unaltuna:** None.

E-P09.06

An Andermann syndrome case of Bulgarian Roma origin due to a new frameshift mutation in the *SLC12A6* gene

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Andermann syndrome [OMIM: # 218000] is an autosomal recessive disease which is a characterized with motor and sensory neuropathy, mental retardation, facial dysmorphism, developmental delay, muscular hypotonia, seizures etc. Most cases also present with agenesis or malformation of the corpus callosum. Andermann syndrome is caused by mutations in the *SLC12A6* gene [OMIM:*604878]. Here we report a male infant from Roma origin at the age of 8 months. The clinical symptoms include muscle hypotonia, areflexia and agenesis of corpus callosum on brain ultrasound. Furthermore, the patient has developmental delay, with the greatest impairment found in his motor functions. Interestingly, he presented clinically like spinal muscular atrophy plus syndrome. Based on a previously exercised SMA genetic test with a negative result, Sanger sequencing of the *SLC12A6* gene was performed. A novel homozygous deletion was found - c.2604delT, p.(Asp868GlufsTer11) clarifying the patient's case as Andermann syndrome. The variant has not previously been reported in patients with similar clinical manifestations. Segregation analysis in the family showed the same variant in heterozygous state in the patient's parents, sister and brother. Based on the variant type which implies a functionally inferior protein product the variant is probably pathogenic. In conclusion, we present the first clinically diagnosed and genetically verified patient with Andermann syndrome in Bulgaria so far. We established a new mutation in the *SLC12A6* gene in a patient of Roma origin, born to non-consanguineous parents, probably a private variant for the Bulgarian Roma (Gypsy) population?

T. Todorov: None. **I. Pacheva:** None. **Z. Halil:** None. **F. Galabova:** None. **R. Yordanova:** None. **I. Geneva:** None. **I. Ivanov:** None. **A. Todorova:** None.

E-P09.07

Val34Leu polymorphism in Factor XIII is associated with low risk of Aneurysmal subarachnoid haemorrhage in a South Indian population

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Background: The rupture of brain aneurysm causes bleeding in the subarachnoid space and is known as aneurysmal subarachnoid haemorrhage (aSAH). In our study we evaluated the association of *Factor XIII* polymorphism and the risk of Aneurysmal subarachnoid haemorrhage (aSAH) in a South Indian population.

Methods: The study was performed in 200 subjects with aSAH and 205 healthy control subjects. Five ml blood samples were collected from subjects and DNA isolated were used for genotyping of rs5985 (Val34Leu) polymorphism of *Factor XIII* with Taqman® allelic discrimination assay. Statistical software R.3.0.11 was used to analyse the data and P value <0.05 was considered as statistically significant.

Results: In our study, *Factor XIII* Val/Leu variant genotype frequency was higher in control subjects (18%) compared to aSAH patients (9%). Val34Leu variant showed significant difference in genotypes (χ^2 =5.81 ; df=2 ; P = 0.04) and allele frequencies (χ^2 =4.12 ; df=1 ; P = 0.04) between cases and controls. Val/Leu genotype (OR=0.48, 95%CI=0.26-0.88, P=0.02) and Leu allele was significantly associated with low risk of aSAH (OR=0.55, 95% CI=0.32-0.95, P=0.03). Significant association of genotypes was observed in dominant model (OR=0.50,95% CI=0.28-0.90, P=0.02). In subtyping, we found Leu/Leu genotype was associated with Basilar top aneurysm (OR=3.59, 95%CI=1.1111.64, P=0.03).

Conclusion: These results suggest that *Factor XIII* Val34Leu polymorphism is associated with lower risk of aSAH in South Indian population.

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E-P09.08

A new case involving 2q13 microduplication associated with autism spectrum disorder, intellectual disability and dysmorphic features

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by impairment of social interaction, reduced communication skills and stereotyped patterns of behavior. The etiology of ASD is complex including genetic, epigenetic and environmental factors. 2q13 duplication have been associated with developmental delay, intellectual disability, ASD and dysmorphism. Several previous studies have linked the NPHP1 gene, located in this genomic region, to ASD. In this paper we report a new case of 2q13 duplication in association with ASD and intellectual disability. The patient is a 5 years old boy born at term from health, non-consanguineous parents who was referred to the department of Child Psychiatry for speech delay and behavioral problems. Clinical evaluation revealed dysmorphic features (high forehead, large protruding ears, open-held mouth), severe speech delay (he says only 6 simple words), moderate intellectual disability, autistic behavior (no visual contact, difficult social interaction, stereotypic movements). The genomic profile obtained by array-CGH (Agilent platforms) unveiled a 645 Kb duplication at 2q13 (110457697-111103309, hg18) encompassing *RGPD6*, *MALL* and *NPHP1* genes. To date, 11 cases with 2q13 microduplication, including *NPHP1* and *MALL* genes, have been reported. All patients presented behavior problems including ASD, attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder. Intellectual disability was also noted in 8 patients. Other features described in some cases were speech delay and dysmorphic traits. All these findings were found in our case, further contributing to the delineating of the 2q13 microduplication.

I.O. Focsa: None. **I. Streata:** None. **S. Sosoi:** None. **M. Ioana:** None. **R. Grozavescu:** None. **M. Budisteanu:** None.

E-P09.09

Duplication of 15q11.2 BP1-BP2 region in Bulgarian autistic patient

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It is well known that copy number variants (CNVs) with different frequency and inheritance patterns contribute to neurodevelopmental disorders etiology and could be considered as significant risk factors. Here we report autistic behaviour patient with lack of speech and developmental delay that was referred for genetic testing. Both father and brother of the proban are heterozygous carriers of beta-talasemia mutations. The patient was previously tested for Fragile X-syndrome and X-linked mental retardation related genes were sequenced as well as whole exome sequencing was performed without detecting any relevant variants correlating with the clinical features. The only positive result was for HLA DQ2 and HLA DQ8 genes predisposing to celiac disease. At the end aCGH array (180K) was performed and it detected 278kb duplicated region (11p14.3 / chr11: 22102205-22361578) that included the *ANO5* and *SLC17A6* genes. This duplication was interpreted as uncertain clinical significance based on the international recommendations. Second aCGH analysis with higher resolution (2,7M) was perform so we can gain additional information and eventually to clarify the previous finding. An additional duplication of 312 kb was detected in chromosome 15 (15q11.2), which included 4 genes: *TUBGCP5*, *CYFIP1*, *NIPA2* and *NIPA1* (PMIDs: 28588435, 27566550). Duplication was inherited by the father who is without clinical manifestation. The 15q11.2 BP1-BP2 region is found duplicated or deleted in people with cognitive, language, and behavioral impairmen and a variable phenotype so probably this finding is the leading cause for the clinical features in the affected child.

A. Mandadzhieva: None. **A. Kirov:** None. **I. Pacheva:** None. **E. Simeonov:** None. **T. Todorov:** None. **A. Todorova:** None.

E-P09.10
Metabolism of vitamin D system in children with autism spectrum disorders

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Introduction: The frequency of autistic spectrum disorders (ASD) over the past 5 years has increased from 1:110 to 1:68 and continues to grow. An important role in etiopathogenesis of ASD is given to metabolic disorders, in particular, to the disorder of metabolism of vitamin D system, which plays one of the leading roles in maintaining the epigenetic health of the organism.

Purpose: To study the state of vitamin D metabolism in children with ASD for the development of the examination and treatment algorithms.

Results: We examined 130 children: 86 with ASD and 44 neurotypical. The polymorphism Bsml of the VDR gene was studied: bb (pathological homozygote) - 13 (15.20%) and 0 (0%), respectively, Bb - 43 (50.00%) and 26 (59.09%), BB - 29 (33.72%) and 18 (40.90%). 95% of children with ASD showed a decrease of 25-OH-D level in the blood, whereas in the control group in only 9% cases. Polymorphic variants of the genes methylation cycle were studied: an increase in the frequency of MTHFR 677 C/T polymorphisms (47.67% and 36.36%, respectively), MTHFR 677 T/T (9.30% and 6.82%), MTRR 66 G/G (31.40% and 25.00%), MTR 2756 A/G (44.19% and 34.09%).

Conclusions: The specific weight of vitamin D deficiency in children with ASD occupies one of the leading places and frequent combination with a disorder of the methylation cycle allows to carry out effective complex therapy.

S. Lisniak: None. **Y. Grechanina:** None.

E-P09.11
Identification of 4q24 microdeletion in a child with autism spectrum disorder, epilepsy and postaxial polydactyly

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Introduction: Identification of rare CNVs leads to the great difficulties in evaluating the clinical significance of these chromosomal changes, and this is an important part of genomic research.

Materials and Methods: We examined a 6-years old male patient with a mild dysmorphic features: short palpebral fissures, long eyelashes, large ears with fleshy lobe and outward turned helix, hyperlaxity of joints. The boy is only child of non-consanguineous healthy parents with no history of congenital anomalies or developmental delay. The boy was born with uses of vacuum extraction after 41 weeks of an uneventful pregnancy. At birth, he presented macrosomia (4230g/57cm, head circumference 37cm), bilateral postaxial polydactyly and stridor. Developmental delay presented from early stages, later he demonstrated poor speech. He was diagnosed autism spectrum disorder at the age of 3 years. At the age of 5 years he demonstrated subclinical epileptiform activity on EEG.

Results: Chromosomal microarray analysis using Affymetrix Cytoscan 750k array revealed a 6 Mb microdeletion

at 4q24 region (arr[hg19] 4q24(101574821_107631432) x1). Due to bioinformatics assay deletion was classified as variant of unknown significance. However, deletions comprising the region 4q24 have recently been described among individuals with an overlapping phenotype. The CXXC4 gene located in deleted region might be a candidate gene for postaxial polydactyly, as it inhibits the Wnt-signaling pathway and subsequently transcription of GLI3 gene, which is associated with polydactyly.

Conclusions: We assume that the 4q24 locus is associated with a combination of polydactyly and mental retardation. Finding candidate genes associated with cognitive impairment is a potential target for further investigation.

O. Novoselova: None.

E-P09.12

Analysis of *ATXN1* and *ATXN2* repeat length in *C9ORF72* expansion carriers

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Introduction: Phenotypic spectrum of *C9ORF72* hexanucleotide expansion, besides amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), is widening to other neurodegenerative disorders such as Alzheimer's disease (AD) and Huntington disease like (HD like) syndrome. Latest studies hypothesize possible role of CAG repeats of *ATXN1* and *ATXN2* gene in phenotypic expression of *C9ORF72* expansion carriers.

Materials and Methods: A large cohort of Serbian patients diagnosed as ALS (311), FTD (276), AD (170), HD like (145) was analyzed to determine the number of *C9ORF72* repeats using standard PCR amplification with one fluorescently labeled primer followed by fragment analysis on capillary electrophoresis. Repeat-primed PCR was performed for apparently normal homozygous samples. In addition, all *C9ORF72* expansion carriers were screened to estimate the number of CAG repeat length in *ATXN1* and *ATXN2* using one fluorescently labeled primer and sized by capillary electrophoresis.

Results: The presence of hexanucleotide repeats was detected in 12 ALS (3,86%), 5 FTD (1,81%) and 1 HD like (0,7%) patients. One (0,59%) AD patient had borderline repeats number. In majority of *C9ORF72* expansion carriers *ATXN1* and *ATXN2* repeat length was normal. The most frequent *ATXN1* allele was with 29 repeats and the most frequent *ATXN2* allele was with 22 repeats. Intermediate repeat length allele of *ATXN1* (35 repeats) was detected in only one ALS patient. Intermediate repeat length of *ATXN2* (27 and 28 repeats) was detected in 2 ALS patients.

Conclusion: To elucidate the significance of *ATXN1* and *ATXN2* intermediate repeats among ALS *C9ORF72* carriers further analysis needs to be conducted.

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E-P09.14

Further delineation of ACPHD syndrome and a novel mutation in DNAJC3

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We recently identified two siblings from consanguineous parents who shared similar phenotypic features. These included motor and mental retardation, cerebellar ataxia, neuropathy, bilateral hearing loss, endocrine changes and some congenital anomalies including microcephaly, facial asymmetry, tubular nose, prominent ears. We performed chromosomal microarray analysis and whole exome sequencing on the family to identify the genetic cause. No copy number changes were detected, but we identified a p. Arg415Pro homozygous mutation in DNAJC3 gene in both affected siblings. This mutation was predicted to be damaging and was not present in Exac or Kaviar databases. DNAJC3 is a co-chaperone of BiP, an endoplasmatic reticulum member of the HSP70 family, which promotes normal protein folding. Homozygous mutations in the DNAJC3 gene were recently identified as being causative for combined cerebellar and peripheral ataxia with hearing loss and diabetes mellitus (ACPHD) syndrome [OMIM #616192] and since then one patient with hypothyroidism in addition to ACPHD has been reported. The clinical features of the patients were consistent with ACPHD syndrome, but are now further expanded to include growth hormone and thyroid hormone deficiencies and mild dysmorphic features. Given the crucial role of DNAJC3 in endoplasmatic reticulum protein folding, the clinical phenotype of the patients with DNAJC3 mutations may correlate with the overlapping features of the unfolded protein response diseases like

Marinesco-Sjogren syndrome, Wolcott-Rallison syndrome, and Wolfram syndrome type 1.

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E-P09.15

An Iranian patient with Charcot Marie Tooth type 2 caused by a novel *GDAP1* mutation

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Introduction: Charcot Marie Tooth Type 2 (CMT2) is a type of CMT which is characterized by distal limb muscle weakness and atrophy.So far more than 20 different genes have been identified for CMT2.

Materials and Methods: A 4-year-old boyfrom an Iranian consanguineous family with muscle weakness, walking difficulty, severe peripheral polyneuropathy, areflexia and kyphoscoliosis, referred to Kawsar Human Genetics Research Center (KHGRC), was investigated in this study. Whole exome sequencing was used to find the genetic cause of the disease. Sanger sequencing was performed to confirm the NGS finding and genotyping the parents.

Result: A novel homozygous splice site mutation (c.579 +5G>C) in *GDAP1*gene was identified in the proband. The results of family segregation analysis and in silico study showed that this mutation cancause CMT disease type 2.

Discussion: Here we report a new mutation in the *GDAP1* gene in an Iranian patient with CMT2. Despite the importance of the study on genetic causes of CMT in countries with high rate of consanguineous marriages, only limited studies have been performed on the molecular genetics of the different types of CMT in Iran. It is clear that identification of common causes of CMT in Iranian population can be helpful for genetic counseling and designing a cost effective molecular diagnostic algorithm for this disease in Iran.

Keywords: Charcot Marie Tooth Type 2, *GDAP1*gene, novel mutation

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E-P09.16

Behavioral phenotype related to CNV in 2q13: report of one patient affected by duplication and a patient carrying a deletion

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Two unrelated patients with behavioral phenotype and a CNV affecting the same region in 2q13 are here presented: one has a deletion (patient A), and the other a duplication (patient B). Patient A, female, clinical diagnosis: "Borderline intellectual functioning + mixed disorder of scholastic skills". Last follow-up at 10+4/12 years: height: 75^{th}-90^{th} pc; weight: 75^{th}-90^{th} pc; head circumference: 51cm; Tanner's scale: B1-P2, thelarche. Dysmorphic features reported: low frontal hairline, low ears implant, hypertelorism, cubitus valgus, right convex scoliosis; signs of developmental dyspraxia.Patient B, male, clinical diagnosis: "pervasive developmental disorder not otherwise specified with speech delay and stereotypies", last follow-up at 10+6/12 years: height: 10^{th} pc; weight: 10^{th}-25^{th} pc; head circumference: 50cm; intermammillary distance: 16cm; Tanner's scale: G2 (testis 4cc). Dysmorphic features reported: asymmetric and long head and face, low frontal hairline, hypotelorism, enophthalmos, deviated nose, thin lips, high-arched palate, unusual voice's pitch, stiff right elbow, 5^{th} finger clinodactyly; right genu valgum; internal foot rotation; no scoliosis. SNP-arrays were obtained at 75kb resolution using a Cytoscan HD. Data was analyzed using ChAS and UCSC's hgLiftOver and genomeBrowser. Both patients are affected by a 492kb CNV in the same region located in 2q13 cytoband. According to ISCN2013 and hg38, result for patient A is arr[hg38]2q13(110116257-110608419)x1, and arr[hg38]2q13(110119299-110611687)x3 for patient B. RT-PCR on *NPHP1* was used to confirm results. Genes involved in both CNVs are *NPHP1*, *LIMS3*, *RGPD5*, and *RGPD6*; there are also a miRNA, 3 LOCs and a lincRNA. The CNV present in Patient B also comprises the final part of *MALL*.

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E-P09.17

Search of genetic markers associated with cognitive performance in the elderly by whole exome sequencing: a pilot study

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Cognitive performance is an important endophenotype for various neurodegenerative and neuropsychiatric diseases. The aim of the study was to find new genetic markers, located in the exome, associated with the variability of the cognitive functions in the normal elderly. The cognitive functions were assessed using the Montreal Cognitive Assessment (MoCA) in 710 elderly from Tomsk, Russia. The sample was subdivided into 4 quartiles according to total MoCA score. Subsets of individuals from the 1st and 4th quartiles were subjected to whole exome sequencing using SeqCap EZ MedExome kit which covers approximately 1.5% of the human genome. Four genetic markers located in the introns of three genes (PKD1 on chromosome 16, ATAD5 and DNAH17 on chromosome 17) were found to be exome-wide significantly associated with the cognitive performance. All 4 SNPs as well as their respective genes were not found previously in any association with cognitive functions, neurodegenerative or neuropsychiatric diseases. PKD1 encodes polycystin 1, transient receptor potential channel interacting protein, involved in polycystic kidney disease. Genetic markers in ATAD5, encoding of ATPase family AAA domain containing protein 5, were found associated with height and body mass index in previous GWA studies, while genetic variability in dynein axonemal heavy chain 17 gene (DNAH17) contribute to triglyceride levels. The associations found in this pilot study are now under replicative analysis in a wider sample from the normal elderly population characterized by the battery of cognitive tests. This work was supported by the Russian Science Foundation (project # 16-14-00020).

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E-P09.18

Classic Dravet Syndrome in an adolescent male – case report

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Introduction: Dravet syndrome is a genetic severe myoclonic epilepsy of childhood with worldwide birth prevalence is <1/40,000. Affected children present from the first year of life prolonged febrile and non-febrile, generalized, clonic or hemiclonic epileptic seizures. 85-90% of Dravet syndrome cases are due to a mutation or deletion in the *SCN1A* gene. This disorder can be autosomal dominant inherited but most are due to de novo mutations. Moderate to severe cognitive impairment and intractable epilepsy is common. Diagnosis is based on clinical and electroencephalographic (EEG) findings. The most common cause of death is status epilepticus.

Materials and Methods: We present a boy aged 12 years and 7 months diagnosed from the first year of life with Dravet syndrome. The patient's clinical work-up included neurologic, functional and imagistic assessment (brain MRI, electroencephalogram). SCN1A gene sequencing was performed.

Results: Our patient has often been hospitalized for severe recurrent febrile seizures, some accompanied by apnea requiring orotracheal intubation and assisted ventilation. The patient presents daily partial seizures under antiepileptic treatment and tonic-clonic febrile generalized seizures during upper-tract respiratory simple infections. He shows developmental regression (moderate cognitive impairment, lack of coordination, hyperactivity, difficulty in relating to others), restless sleep and requires constant care from his mother. The electroencephalogram showed abnormal activity. *SCN1A* sequencing revealed c.4970_4971insATCG, p.Thr1658SerfsX14, a not yet reported small insertion.

Conclusions: This is a typical case of Dravet syndrome with difficult seizure management. The condition severely impacts the patient's and family's quality of life and the long-term prognosis is poor.

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E-P09.19

Novel homozygous KCNJ10 mutation in a patient with non-syndromic early-onset cerebellar ataxia

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Mutations in KCNJ10, which encodes the inwardly rectifying potassium channel Kir4.1, a primary regulator of membrane excitability and potassium homeostasis, cause a complex syndrome characterized by seizures, sensorineural deafness, ataxia, intellectual disability and electrolyte imbalance called SeSAME/EAST syndrome. We describe a 41 years old patient with non-syndromic, slowly progressive early-onset ataxia. Targeted next generation sequencing identified a novel c.180T>G (p.Ile60Met) missense homozygous mutation. The mutated residue Ile60Met likely impairs phosphatidylinositol 4, 5 bisphosphate (PIP2) binding which is known to play an essential role in channel gating. Our study expands the clinical and mutational spectrum of KCNJ10-related disorders and suggests that screening of this gene should be implemented in patients with early-onset ataxia, with or without syndromic features.

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E-P09.20
Heterozygous premature stop mutation in GAL gene may not the cause of epilepsy

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Introduction: Epilepsy has a complex etiology. Despite evidence for the participation of genetic factors, the genetic basis of epilepsy remains largely unknown. Here we are presenting a case of a 8 year old girl, with healthy parents and with epilepsy and mental retardation. Dravet syndrome was rule out before our examination.

Materials and Methods: Total genomic DNA was extracted from her blood sample and first analyzed with a comprehensive epilepsy gene panel test, which contains 200 most relevant genes for epilepsy. Capture based library preparation (GeneSG) was used. Second, exome sequencing was performed using Agilent SureSelect V5 kit coupled with Illumina HiSeq sequencing. Sequencing reads were mapped to the reference genome (hg38) and after variant calling the variants were classified based on ESP, ExAc, ClinVar and HGMD information.

Results: Gene panel test identified a heterozygous variant with uncertain significance in the AMT gene (c.893C>T) suggesting a possible diagnosis of glycine encephalopathy, but clinical tests became negative. Exome sequencing revealed a heterozygous likely pathogenic premature stop codon in GAL gene (c.7C>T). GAL gene is linked to familial temporal lobe epilepsy (AD). The minor allele frequency of the T allele is 0.00006 in ExAC. Family analysis discovered that, the healthy father is a carrier of this mutation.

Conclusions: The heterozygous premature stop codon mutation of the GAL gene may not the cause of epilepsy.

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E-P09.21
EXOSC3 mutation in infant with hypotonia and cerebellar hypoplasia

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A 2,5 months-old infant girl, was admitted to the ED in a pre-arrest state and was resuscitated successfully, although she continued to present a very important respiratory distress syndrome that demanded intubation and mechanical ventilation subsequently. The baby presented microcephaly, generalized hypotonia with present tendon reflexes, blepharoptosis and nystagmus with no eye contact, no reaction to stimuli, and failure to thrive. From her medical history, she was a preterm baby (35w), IUGR (weight of birth 2180g), from healthy parents, who presented hypotonia at birth and had been hospitalized at the NICU for feeding difficulties and swallowing insufficiency. She had also one more hospitalization because of reduced feeding, vomiting and suspicion of aspiration. Infection and metabolic screening, karyotype and gene test for Prader-Willi were performed and were negative.

Cerebral MRI indicated an important cerebellar hypoplasia with a predominant frontal lobe, hypoplasia of middle cerebellar peduncles and vermis, as well as communication of the 4th ventricle with mega cisterna magna.

During the last hospitalization at the PICU, the patient passed away due to severe respiratory failure and cardiorespiratory arrest. A whole exome sequencing had already been demanded, that identified an homozygous p.

Gly31Ala mutation in EXOSC3, posing the diagnosis of Pontocerebellar hypoplasia type 1B. The p.Gly31Ala mutation is well correlated with the severe phenotype of the disease expressed in our patient, as well as with her Romani origin. A blood sample from the mother of the baby was requested and the analysis is in progress, in order to provide genetic counseling.

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E-P09.23

Detection of inborn errors of metabolism (IEMs) in patients with psychosis using NGS

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Inborn errors of metabolism (IEMs) are a group of genetic disorders involving abnormalities in biochemical processes. The majority of IEMs are caused by defective enzymes, which often results in accumulation of toxic upstream substances leading to problems. IEMs are considered as rare diseases occurring in less than 1/100.000 live births and presenting during early childhood. The true incidence is thought to be much higher because of under recognition of clinical manifestations, especially as symptoms can be quite aspecific and diverse. It is hypothesized that patients with psychotic disorders can be present in the under diagnosed group of IEMs. It is important to recognize the (neuro) psychiatric presentations of IEMs, since some IEMs are easily treatable and since treatment is more efficacious at early stages of psychiatric manifestations, before irreversible neurological damage occurs. Unfortunately, diagnosis is often missed or delayed resulting in poorer prognosis. The objective of this study is to investigate the diagnostic yield of a NGS based gene panel for neurodegenerative metabolic disorders into a clinical psychiatric setting. We aim to detect rare, treatable organic causes of psychotic disorders in patients at ''high risk''. We're fully analyzing the coding regions of 67 IEM genes using a type-A gene panel in 100 patients with, preferably, a psychotic disorder and additional criteria such as cognitive decline, treatment resistance or neurological symptoms. In addition, we provide pharmaco genetic information by genotyping the most relevant anti-psychotic metabolizing sequence variants in cytotochrome p450 genes.

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E-P09.24

A case with Joubert syndrome with a new mutation in the CEP290 gene

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Introduction: Joubert Syndrome is a rare genetic disease with wide clinical and genetic heterogenity. The ''molar tooth sign' at the neuroradiological imaging, hypotonia and developmental delay are essential features for diagnosis of this disease. Up till now, 34 different genes have been identified and most of these demonstrate autosomal recessive pattern. Here, two mutations on CEP290 gene, one of which we firstly reporting in literature, are presented.

Material-Methods: 7 months old female patient admitted to our clinic with developmental delay and upward motion of eyes. She was second child of an unrelated parents. She was born at the 43th weeks of gestation with vaginal delivery and she showed no complication postnatally. At the physical examination wide-prominent forehead, rotatory nystagmus, left upper eyelid ptosis, hypertelorism, anteverted nares, wide philtrum and inverted nipples observed. Fundoscopy revealed hypopigmented regions at the retina. Cranial MRI showed 'molar tooth sign', characterized by dysplastic appearance of vermis and cerebellar hemispheres.

Results: Karyotype analysis was 46,XX. Next generation sequence analysis of CEP290(NM_025114) gene showed mutations of c.5493delA and c.5975_5976delGA. The variant of c.5493delA was previously reported as pathogenic at ClinVar and HGMD databases, whereas c.5975_5976delGA variant was not reported at neither of these databases. Because of frameshift nature of this deletion and patient's consistent phenotype with Joubert syndrome, we assume this variation to be pathogenic.

Conclusions: We report a novel mutation in CEP290 gene which may cause Joubert syndrome.

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E-P09.25

A case with Kleefstra Syndrome derived from ring chromosome 9

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Kleefstra Syndrome (KS,OMIM 610253) is a rare neurogenetic disorder estimated to affect at least 1:200,000 individuals. KS is most commonly caused by deletion in the 9q34.3 chromosomal region that includes *EHMT1* gene and characterized by intellectual disability, childhood hypotonia, and distinctive facial features (arched eyebrows, midface hypoplasia, anteverted nares, full everted lower lip). Also clinical features include congenital heart and urogenital defects, epilepsy, behavioral and psychiatric disorders. The proband, a 2 years old girl, was the second-born child of nonconsanguineous marriage. She was born at term weighed 2500 gr. On examination, facial dysmorphic features including arched eyebrows, low-set ears and thickened ear helices, microcephaly, thickened lips were observed. Cytogenetic analysis revealed ring chromosome formation derived from chromosome 9. The karyotype was interpreted as 46,XX,r(9)(p24q34). DNA microarray analysis was performed by Affymetrix Cytoscan Optima (Affymetrix, USA) and the result was arr[hg19] 9p24.3p24.1 (203,861-8,348,602)x3 arr[hg19] 9q34.3(140,719,389-141,020,389) x1. Here we present a girl that present distinctive facial features with Kleefstra Syndrome resulted from ring chromosome 9.

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E-P09.26

Leptin receptor gene variant rs1137101 is associated with multiple sclerosis onset age

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Introduction: Changes in leptin receptor (LEPR) activity are related to pathogenesis of multiple sclerosis (MS). MS is a complex neurological disease whose prognosis depends on various factors such as disease onset age, gender, type of neurological dysfunction, etc. LEPR gene variant rs1137101 (c.668A>G, p.Gln223Arg) alters receptor binding activity. In this study, we aimed to investigate the association of LEPR rs1137101 gene variant with clinical parameters of MS.

Materials and Methods: The study included 475 patients with relapsing-remitting (RR) MS: female to male ratio=1,6, age(mean±SD)=38,1±10,4 years, disease onset age(mean±SD)=30,8±9,0 years, EDSS(mean±SD)=3,1 ±1,8, MSSS(mean±SD)=4,8±2,4. Genotyping was performed using TaqMan® technology. Statistical analysis was done using SPSS software (SPSS 17.0).

Results: We found a significantly different distribution of rs1137101 genotypes by recessive model, GG vs. AA+AG, according to MS onset age cutoff of 30 years (MS onset age ≤ 30: AA+AG=75,6% and GG=24,4%, MS onset age ˃ 30: AA+AG=84,9% and GG=15,1%; Fisher's exact test p = 0,015). Carriers of A allele had a more frequent MS occurrence after the age of 30, in comparison to GG genotype carriers, and this association remained significant after the adjustment for gender (adjusted OR=1,82, ±95% CI=1,13-2,91, p = 0,013).

Conclusions: LEPR gene variant rs1137101 is associated with onset of RR MS in an age-dependent manner. The current finding should be verified in a larger study group.

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E-P09.30

MYH7-related myopathy in a Bulgarian family: a novel splice acceptor variant in patients with late onset clinical manifestation established by NGS

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A patient from Bulgaria with suspected clinical diagnosis of Limb-girdle muscular dystrophy type 1A was tested for mutations in the *MYOT* gene [OMIM *604103], unfortunately with a negative result. The patient has a positive family history - father, two aunts and sister with the same

clinical manifestations. Based on the clinical symptoms of the patent: muscle pain in the legs and hands, difficulty in climbing stairs, EMG which showed myogenic damage, we performed NGS (Illumina TruSight One). Targeted evaluation of genes associated with the clinical symptoms of the patient showed a heterozygous splice acceptor variant c.5560-2A>C (NM_000257.3) in *MYH7* gene. The detected variant was confirmed by Sanger sequencing. c.5560-2A>C is not recorded in the database dbSNP and was not present among 60000 controls subjects of the ExAC project. The detected variant is predicted to result in an in-frame deletion of exon 38 of the MYH7 transcript. A variant with similar effect was identified in patients with MYH7-related myopathies. Segregation analysis of the family showed the same variants in the patient's sister and her two children (who do not have clinical presentation at the moment). The mother of the patient with a congenital heart defect is not a carrier of the detected variant. In conclusion, we present a novel splice acceptor variant which can lead to skipping of exon 38 of the *MYH7* gene. The detected variant was established only in the family members with muscular clinical manifestations problems.

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E-P09.31

CNVs associated with autism spectrum disorder in a cohort of children from Goiás (Brazil)

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Introduction: Autism spectrum disorder (ASD) is a neurodevelopmental disorder with a complex genetic architecture. The worldwide prevalence of autism is increasing. However, autism prevalence is low (0.27%) in Brazil due to misdiagnosis of the disorder.

Material and Methods: We report on 16 probands with ASD referred by the public health system of Goiás. ASD assessment using Autism Diagnostic Interview-Revised (ADI-R), G-band karyotyping, and PCR of *FMR1* using Amplidex® kit were carried out for all patients. Chromosomal Microarray Analysis (CMA) using GeneChip® CytoScanHD™ array was done for patients who had normal results in karyotype and PCR.

Results: The behavioral phenotypes for all participants were classified in the ASD according to ADI-R results whose karyotypes showed neither visible numerical nor structural chromosomal aberrations. *FMR1* analysis revealed two children affected by Fragile-X Syndrome. CMA showed a total of 21 CNVs identified in 12/14 (86%) patients. A total of 13/21 pathogenic CNVs were observed in 8 patients, all located at genetic ASD hotspots, including 22q11.23, Xp22.33 (2/13), 16p11.2 (3/13), harboring no OMIM genes. However, the hotspots at 15q13.3, 15q11.2 (2/13), and Xp11.23 (2/13) harbored *CHRNA7*, *PWRN2* and *ZNF630* genes, respectively, associated with ASD. The remaining CNVs were classified as having unknown clinical significance, distributed at loci: 2q12.2, 3p22.1, 9p21.1 (3/8), Xp22.32, Xq21.1, and Xq24. Two patients showed no alterations in CMA.

Conclusions: Identify CNVs and genes using CMA in our patients was very important to understand the heterogeneous spectrum of ASD. The results helped the clinical management of all patients.

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E-P09.32

Rupture of abdominal aortic aneurysm and renal failure in an adult patient with undiagnosed Neurofibromatosis type 1 (NF1)

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Introduction: Neurofibromatosis Type 1 (NF1) is an autosomal dominant disorder affecting 1/ 3000 individuals and caused by SNVs, deletions and duplications affecting the *NF1* gene. Vascular lesions of medium and large size arteries and veins are a well recognized, albeit rare, manifestation of NF1. We report on an adult patient

retrospectively diagnosed (clinically and molecularly) with NF1 after surgery for a ruptured abdominal aortic aneurysm and renal failure.

Materials and Methods: A 37 year old female patient was admitted for emergency surgery due to a ruptured abdominal aortic aneurysm, renovascular hypertension and renal failure. Signs of NF1: multiple café-au-lait macules, axillary fleckling, multiple cutaneous neurofibromas, and an external vaginal plexiform neurofibroma, became evident upon patient examination. DNA was extracted from peripheral blood and was analyzed by NGS with a customized *NF1* gene panel (QIAseq, Qiagen GmBh, Hilden Germany) that covers 100% of the coding exons, as well as the intron-exon boundaries. Additionally, Chromosomal Microarray Analysis (CMA) was done using the high resolution 2x400K G3 CGH+SNP microarray platform (G4842A, Design ID 028081, Agilent Technologies, Santa Clara, CA, USA).

Results: NGS did not reveal a pathological SNV for the *NF1* gene but the CMA revealed a novel duplication covering exons 19-27 of the *NF1* gene [arr[GRCh37] 17q11.2(29553704_29562744)x3].

Conclusions: We have identified a novel *NF1* gene duplication (exons 19-27) in a 37 years old female patient presenting with mild NF1 but spontaneously ruptured abdominal aortic aneurysm. The rare presentation of cases with NF1 vasculopathy could be due to an underappreciation of its recurrence.

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E-P09.33

High Throughput Sequencing identifies *PINK1* p.G47R: A rare mutation identified in a Parkinson's disease patient

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Introduction: Heterozygous mutations in PTEN induced kinase-1 (*PINK1*) reportedly increase risk for late-onset Parkinson disease (PD). PINK1 localises to the mitochondria, recruiting and phosphorylating Parkin leading to mitophagy of damaged mitochondria. Mutations in *PINK1* abolish such effect, increasing the vulnerability of cells to oxidative stress.

Methods: High throughput sequencing (HTS) of PD related genes identified an extremely rare mutation, c. G139C:p.G47R in exon 1 of *PINK1*. The Maltese Geoparkinson collection (158 patients, 378 matched controls) was genotyped for this variant by PCR and RFLP using Hpy166II.

Results: The variant (gnomAD maf < 1:10,000) was identified in heterozygosity in a 73 year old male patient with two affected first degree relatives. p.G47R results in the replacement of glycine, a small amino acid with a non-polar, aliphatic side chain, with arginine, an amino acid with a large positively charged side chain. G47 is found in the mitochondrial transit domain (MTD) of *PINK1*, which is the part of the protein responsible for the transport of PINK1 to the mitochondrion. Data analysis of 101 HTS datasets and PCR-RFLP of the Maltese Geoparkinson collection did not identify any other individual with this variant.

Conclusion: This is the first report of the rare variant p.G47R in a late-onset PD patient with a family history of the condition.

Funding: Patient data and samples were collected as part of the 5th framework (FP5) EU funded GeoParkinson study, project number QLK4-CT-1999-01133. HTS was funded through the NGS Project funded by the National Research and Innovation Programme (2012).

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E-P09.35

Novel *COL4A1* mutation in a fetus with early prenatal onset of schizencephaly

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Introduction: Schizencephaly is a congenital anomaly characterized by an abnormal gray matter-lined defect extending from the pial surface to the lateral ventricles. Moreover, porencephaly is a congenital brain disorder characterized by cavitation or a cerebrospinal fluid-filled cyst in the brain without gray matter lining. To date, mutations in *COL4A1* have been reported to cause porencephaly or schizencephaly. Here, we report a Japanese patient with schizencephaly, determined by serial fetal ultrasonography, fetal magnetic resonance imaging, and *de novo* novel mutation in *COL4A1*.

Materials and Methods: To investigate the molecular basis of a Japanese patient with schizencephaly, genomic DNA from the patient and his parents was isolated from peripheral blood by standard protocols after receiving written informed consent. Genomic DNA was captured

using the TruSight One Sequencing Panel (Illumina, Inc., San Diego, CA, USA). Captured DNA was sequenced on a MiSeq platform (Illumina) with 151 bp paired-end reads.

Results: Average coverage depth of the entire panel was 58.51 reads, with 97.7 % of targeted bases covered at 10× sequence reads. Targeted resequencing identified a novel heterozygous mutation in *COL4A1*, which is a known schizencephaly-causing gene (NM_001845; c.2645G>A and c.2646C>A, p.Gly882Glu). Sanger sequencing confirmed this variant occurred as a *de novo* event.

Conclusions: Our report may be useful for determining the mechanism and developmental process of *COL4A1*-related disease.

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E-P09.36

Contribution of genetic variants in the cognition of Mexican patients with schizophrenia: a pilot study

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Introduction: Cognitive impairment is a cardinal symptom of schizophrenia (SZ), relevant for prognosis and functional capacity of patients. Variants of *COMT, PRODH* and *DISC-1* are risk factors for SZ and individually associated with executive-functions and social-cognition. Their combination may have epistaxis with significant effects on affected brain regions in SZ. Herein, we describe a cognitive-molecular evaluation in Mexican SZ patients.

Material and Methods: 9 variants of *COMT*, 15 of *PRODH* and 4 of *DISC-1* were genotyped in 150 patients and 150 controls matched for age-gender-ethnicity. The association between alleles/genotypes/haplotypes and the score in cognitive domains was evaluated by the battery MATRICS in a subgroup of 40 participants/group.

Results: p.Val158Met *COMT* ($p = 0.0006$, OR=2.80, IC95%=1.54-5.09); p.Asp426Asn *PRODH* ($p = 0.02$, OR=1.58, IC95%=1.04-2.42) and p.Ser704Cys *DISC-1* ($p = 0.0198$, OR=1.8, IC95%=1.68-1.92) were associated with SZ. Risk alleles of *COMT* and *PRODH* in this subgroup were related to lower scores in MATRICS ($p<0.01$). The multivariate analysis suggested that *COMT* variants might participate in perception and management of emotions in SZ patients (c.1-98A>G); while p.Val158Met and c.-225T>C were associated with lower scores in attention processes. The allele Val158 of COMT degrades catecholamines faster than the risk allele and has been related to efficiency in attention tasks and other cognitive abilities.

Conclusions: variants of these genes were confirmed with SZ risk. *COMT* variants were associated with alterations in tests assessing social-cognition and working-memory in SZ patients, but these results should be later confirmed. COMT could be a marker of cognitive impairment and candidate for therapeutic interventions in SZ patients. CONACyT_233695.

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E-P09.37

Association study of *COMT* and *MAOA* genes polymorphisms on a risk of schizophrenia development in the Southern Russian patients

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Introduction: For a long time, the dysregulation in dopamine signaling was considered a major mechanism of schizophrenia development. It is proposed that the increase of dopamine production results in a psychiatric phenomenon called aberrant salience, which forces the brain to detect various external stimuli as important or life-threatening. Polymorphisms of the dopamine metabolism genes *MAOA* and *COMT* were studied in order to assess this hypothesis.

Materials and Methods: The patient group consisted of 100 male and 98 female individuals who suffered from schizophrenia. The control group consisted of 71 individuals. Genomic DNA was extracted from whole blood samples. The potential influence of the rs4680 *COMT* gene polymorphism and the MAOA-LPR polymorphism on schizophrenia development was studied using PCR.

Results: The study showed a lack of any significant differences between both groups studied in regard to the Val158Met *COMT* gene polymorphism. In case of the MAOA-LPR polymorphism, there were significant differences between the groups regarding allelic frequencies. The control group exhibited a higher frequency of 4-tandem repeats in the promoter region of the gene ($p<0,001$). In

addition, the female patients demonstrated a higher rate of the 3-tandem repeats polymorphism than other gendered groups ($p<0.05$).

Conclusions: We failed to establish the association of the Val158Met *COMT* gene polymorphism with an increased risk of schizophrenia. On the other hand, the alleles associated with a decreased activity of the *MAOA* gene may potentially contribute to the disorder development. A further research on the dopamine metabolism genes in relation to the schizophrenia development is required.

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E-P09.38
The prevalence of miR-181b & let-7g expression in schizophrenia cases

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Introduction: The aim of this study is to examine the diagnostic competence of miR-181b and let-7g blood levels in schizophrenia cases.

Materials and Methods: 60 patients who are diagnosed in psychiatry clinic in Bilecik State Hospital were included in the study. In addition, 6 people who are diagnosed with schizophrenia belonging to 3 different families are included in the study group. RNA isolation, cDNA synthesis and qRT-PCR steps were performed with Qiagen branded kits based on SYBR Green method, using the protocol of the commercial company. For statistical analysis, -ΔCt values, obtained from the use of ce-miR-39 Ct values in normalization, were used. Differences between groups were tested by independent sample t test and variance analysis. ROC analysis was performed to calculate the diagnostic competence. "Fold change" calculations were performed online on Qiagen webpage. In all analyzes, alpha error level was accepted as 0.05.

Results: The results are summarized in Table 1

Compared Groups	miRNA	P value	Fold Change	AUC	Specificity	Sensitivity
Schizophrenia (54 patients)	miR-181b	**<0.001**	-7.89	0.817	89%	72%
let-7g	0.895					
Family (6 patients)	miR-181b	**<0.001**	-9.75	0.880	93%	86%
let-7g	0.746					

Conclusion: We have observed that only miR-181b differ in schizophrenia cases. It is also important that miR-181b upregulation found to be significant in all family cases. Significantly, these changes in miRNA expression may have the potential of new biomarkers and this miR-181b upregulation in schizophrenia may also provide the basis for new clinical diagnostics.

S. Eroglu: None. **O. Eroglu:** None.

E-P09.39
The identifying in *SCN1A* gene a novel variants associated with epilepsy

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Background: Mutations in *SCN1A* gene are the most common cause of GEFS+ (generalized epilepsy with febrile seizures plus), SMEI/Dravet syndrome (severe myoclonic epilepsy of infancy). Currently more than six hundred clinically important variants are known in *SCN1A* gene (710 pathogenic in according ClinVar database).

The aims of study: Here, we report mainly for a novel missense and nonsense mutations found in *SCN1A*, associated with SMEI or GEFS+, according to our clinical research study.

Methods: DNA isolation was performed by MagNAPureLC2; 90 biological samples (including probands, parents, a few controls) were tested by NGS (454 GSJunior sequencer (we have conducted research in 2014-2015), NimbleGen SeqCap target enrichment) for genes associated with epileptic encephalopathy (34 genes)). Informed consents were obtained from legal representatives of patients according local ethical approval. All steps of the sample preparation or sequencing were performed in according to the manufacturer's protocols.

Results: We identified a number of heterozygous missense and nonsense mutations (hg19 genome assembly) in *SCN1A* gene: chr2:g.166847972G>A (p.Ala>Val); chr2:g.166848441T>G (p.Ile>Leu); chr2:g.166859155C>T (p.Gly>Ser); chr2:g.166897905A>G (p.Ser>Pro); chr2:g.166904163C>A (p.Asp>Tyr); chr2:g.166909412A>G (p.Leu>Ser); chr2:g.166901591G>A (p.Arg>Ter); chr2:g.166912936C>T (p.Trp>Ter); chr2:g.166930011T>A (p.Lys>Ter).

Conclusions: The authors are grateful to Mds Ananyeva T., Ayvazyan S., Lukyuanova E., and Zhilina S. from Research Center for Children Medical Care for clinical selection of patients. The research was supported by the Department of Health of Moscow (project 2014-2015).

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E-P09.40

A novel variation in SCN2A gene in a patient with Dravet syndrome

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Introduction: Dravet syndrome, also known as severe myoclonic epilepsy in infancy(SMEI), is an epileptic encephalopathy and a component of genetic epilepsy with febrile seizures plus(GEFS+) spectrum. Although pathogenic variants of SCN1A is most common in etiology, plenty of other genes including SCN2A, held responsible for this disease. SCN2A encodes the brain sodium channel NaV1.2 which plays a significant role in generation and propagation of action potential in nerve cell.

Materials and Methods: 13 years old female patient referred to our clinic with epilepsy, intellectual disability and excessive sweating. The patient had febrile convulsion subsequent to routine vaccination at 5 months old. At age 3, she developed afebrile convulsions and followed by anti-epileptics thereafter. Motor milestones were delayed. Her family history was uneventful except death of her father because of colon cancer at 28 years of age. In physical examination; tremor and avoidance of eye contact observed. Cranial MRI and genitourinary USG findings were insignificant.

Results: Sequence analysis using NGS technology, showed no significant variation in SCN1A, SCN1B, GABRG2 and SCN9A. Whereas in SCN2A(NM_001040142), we found a substitution(c.3454G>A, p.Ala1152Thr) that changes amino acid sequence. This variation have not been reported in Ensemble(Grch38) and CLINVAR databases.

Conclusion: Because of biochemical differences between alanine and threonine, it seems likely that this variation could effect secondary and tertiary structure of brain sodium channel NaV1.2 which in turn could end up with phenotype described above. Further studies are needed to determine the exact effect of this variation on severity of disease and prognosis of patients.

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E-P09.41

A novel nonsense variant (Lys177X) of *FGF14* in a Japanese patient with autosomal dominant spinocerebellar ataxia

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Spinocerebellar ataxia 27 (SCA27) is an autosomal dominant SCA caused by variations in the *FGF14* gene encoding fibroblast growth factor 14. We examined a Japanese SCA patient whose deceased father also suffered from SCA. The patient was a 63-year-old male. He had completed junior high school without further education. The chief complaint was slowly progressive dysarthria and gait disturbance those appeared at age 47. He showed pathological saccadic dysmetria, saccadic intrusions into smooth pursuit eye movements, dysarthria, and limb and truncal ataxia. There was a wide-based gait without cane. Limb muscle strength was intact. Deep tendon reflexes were normal or slightly reduced. Pathological reflexes were not shown. He demonstrated mildly impaired vibration sense in the lower limbs. There were no urinary dysfunctions. Brain MRI showed cerebellar atrophy without brainstem involvement. We first confirmed the absence of repeat expansion in known genes responsible for SCAs1-3, 6-8, 12, 17 and dentatorubral-pallidoluysian atrophy. By the exome analysis, we identified a novel heterozygous missense variant (NM_004115, c.529A>T; Lys 177 X) in exon 4 of the *FGF14* gene. FGF14 is known to interact with voltage-gated sodium channels. The variant is expected to generate premature FGF14 proteins lacking the heparin binding site in FGF domain which could modulate the activity of FGF14. We confirmed the absence of the variant in 502 healthy Japanese individuals by Sanger sequencing. There was no record of the variant in ExAC. We conclude that the novel variation in *FGF14* is the causative variant for the SCA27 patient.

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E-P09.42

Genetic and clinical analysis of spinocerebellar ataxia type 36 in a Turkish Family with review of literature

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Expansion of GGCCTG hexanucleotide repeat in the intron 1 of NOP56 was identified as the cause spinocerebellar ataxia type 36 (SCA36), a rare SCA subtype accompanied by motor neuron involvement. First of all, it was determined that these two patients were related to each other. After the affected individuals were identified in their families. In the pedigree analysis, it was found that the disease was transmitted dominantly. The inheritance pattern of the disease has been determined. A neurological examination was performed to patients. Three major findings were identified as a result of the neurological examination performed.1) both upper and lower motor neurons deficit (tongue fasiculation, clonus, babinski extensions)2) loss of muscle power in both upper and lower extremities (3/5) 3) unresponsiveness at cerebellar physicial examination (dysmetry, dysdiadochokinesia). Mild cognitive retardation was detected in the mental examination of the patients. Dysarthria and tongue fasiculation was a severe finding in both patients.As the disease progressed, walking and balance loss increased, resulting in wheelchair movement. Significant enlargement of cerebellar follicles, which were compatible with cerebellar atrophy, was found in brain MR images of patients. In EMG, absence of sensory response was detected with lower motor neuron deficit. Bilateral sensorineural hearing loss was detected in the hearing examinations of the patients and the patient was given hearing aid. Diplopia was detected in the eye examinations performed by the patients. As a result of all these physical examination findings and imaging findings, SCA type 36 was suspected in the patients and the molecular test performed.

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E-P09.43

A novel splice-site variant in *COL11A1* gene - familial case of genetically verified Marshall- Stickler syndrome in Bulgaria

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Stickler syndrome is a hereditary autosomal dominant disorder affecting 1 in 7,500 to 9,000 newborns. The condition is characterized by typical facial, ocular and auditory features. Similar features are also found in Marshall syndrome leading to a continuing debate whether these are distinct entities or different manifestations of a single syndrome. Several mutations causing Stickler syndrome have been found in the *COL2A1* gene, also mutations have been detected in the *COL11A1* causing Stickler and Marshall syndrome. Here we present the first genetically verified case of Marshall- Stickler syndrome in Bulgaria - a familial case spread through at least three generations. The proband is a 2-years old girl with ocular hypertelorism, midface hypoplasia, small saddle nose with flat bridge and craniofacial dysplasia. The proband's father has the same clinical manifestations like the proband, but with a tall and thin stature and mild hearing loss. The same dysmorphology is presented also by the grandfather. Both the father and the proband are clinically diagnosed as Marshall-Stickler syndrome. After a negative *COL2A1* result, we provided a mutation screening of the *COL11A1* gene and found a novel splice-site mutation c.3474+1G>A in intron 45. This variant is related to the clinical presentation in the patient and his father. The variant c.3474+1G>A affects the donor splice site of intron 45 resulting in altered splicing, which leads to production of a nonfunctional protein. The mutation affects the region coding the major triple-helical domain, which represents a mutation "hot spot" for the gene.

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E-P09.45

Whole exome sequencing helps the diagnosis of two siblings with SLC19A3 mutation

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The diagnosis of potentially treatable disorders are very important for the patient and the family. One of these disease is biotin or thiamine responsive encephalopathy type 2. Early usage of the thiamine and biotin has a dramatic clinical effect. Here we report two siblings with early onset encephalopathy and epilepsy. One of them was a boy. He was one and half years old and the other was three months old girl when they were sent to our clinic. Brain MRI

findings of the boy shows the atrophy of frontoparietal and occipital cortex. He had seizures since three months. His sisters MRI findings was active demyelinating neuronal loss. She hasn't got seizures. Their metabolic screening were normal. The parents were first degree relatives. We sent the patients and parents blood for whole exome sequencing. Both of the siblings has a homozygous mutation at SLC19A3 gene p.Lys290Glufs*16(c.623_624insA) . When we look at the parents they were heterozygous carriers. We confirm the results with sanger sequencing. We started their treatment after the results. We are following the patients for two months. Before treatment the brother needed the ventilation machine from his tracheostomy. Now he can breath without ventilation machine. We expect them to be better in time.

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E-P10 Neuromuscular disorders

E-P10.01
Genetic variability of amino acid transporter genes affects physical decline after the fifth decade of life and human survival

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Physical function impairment with increasing age has been associated with substantial morbidity and mortality. Amino acid availability is a rate-limiting factor in the regulation of muscle protein metabolism, and hence a risk factor for age-related decline in muscle performance. Amino acid transporters are emerging as sensors of amino acid availability and activators of mTORC1 signalling, acting as transceptors.

Hence, we sought to evaluate the association of 58 single nucleotide polymorphisms (SNPs) in 10 selected amino acid transporter genes with parameters of physical performance (Hand Grip, Activity of Daily Living, and Walking time). By analysing a sample of 458 subjects aged 50-89 years, we found significant associations with SLC7A5, SLC7A8, SLC36A1, SLC38A2, SLC3A2, SLC38A7 genes. Further investigation of the SNPs in a cross-sectional study including 271 subjects aged 90-107 years revealed associations of SLC3A2, SLC38A2, SLC38A3, SLC38A9 variability with longevity. Finally, a longitudinal study examining the survival rate over 10 years showed age-dependent complexity due to possible pleiotropic effects on different phenotypes for SNPs in SLC36A1, and trade-off dynamics for a SNP in SLC38A9, conferring a survival advantage before 90 years of age and disadvantage later.

On the whole, our findings support the hypothesis that amino acid transporters may impact on the age-related physical decline and survival at old age in a complex way, likely through a mechanism involving mTORC1 signalling.

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E-P10.03
The first patient homozygous for c.197G>T p.G66V mutation in *CHCHD10*

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Introduction: Dominant mutations in *CHCHD10* have been reported to cause a wide range of neurological disorders. The most common *CHCHD10*-related disease in the world is spinal muscular atrophy Jokela type (SMAJ, OMIM #615048), that is caused by mutation c.197G>T p.G66V. SMAJ is a relatively benign adult-onset disorder, characterized by painful cramps and fasciculations affecting the proximal and distal muscles of the upper and lower limbs. The disease is slowly progressive, resulting in weakness and muscle atrophy later in life. In Finland, there are approximately 200 SMAJ patients due to a Finnish founder mutation c.197G>T p.G66V.

Aims: We describe the first patient homozygous for c.197G>T p.G66V.

Results: The onset of the disease was already in childhood and at the age of 36 the patient became wheelchair bound. In contrast to heterozygous SMAJ, muscle biopsy shows clear mitochondrial pathology and the degenerative muscle changes on MRI are extensive. The parents of the homozygous patient both belong to previously known SMAJ families and have a regular SMAJ phenotype.

Conclusions: The phenotype of the homozygous patient is much more severe compared to conventional heterozygous patients. This is the first reported individual in the

world who carries confirmed pathogenic *CHCHD10* mutations on both alleles.

S. Penttilä: None. **M. Jokela:** None. **S. Porre:** None. **O. Raheem:** None. **B. Udd:** None.

E-P10.08
A new case with GLYT1 encephalopathy and sequence variant in the *SLC6A9* gene

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Introduction: Glycine encephalopathy with normal serum glycin (OMIM #617301, also known as GLYT1 encephalopathy) is a very rare autosomal-recessive disorder only reported in a few families of Arab descend so far. The patients usually present at birth with severe hypotonia, respiratory failure and facial dysmorphism. Increased fetal nuchal translucency (NT) and arthrogryposis were previously reported. Homozygous *SLC6A9* mutations (solute carrier family 6 (neurotransmitter transporter, glycine), member 9 or glycine transporter, type 1) are the only known cause of GLYT1 encephalopathy.

Case report: We report on a young healthy and non-consanguineous couple of German origin. They had two consecutive pregnancies with multiple fetal abnormalities. The first fetus was female (46,XX) and presented with an increased NT >6mm, arthrogryposis and polyhydramnion (week 13 and 16). The pregnancy was terminated at 21 +weeks. The fetus in the second pregnancy was male (46, XY) and showed a very similar phenotype with early nuchal translucency (4,8 mm) and severe arthrogryposis on follow-up scans. This pregnancy was also terminated. Exome analysis revealed a homozygous missense variant in the *SLC6A9* gene (c.352G>A,p.Val118Met) in both children, both parents being heterozygous carriers. The variant is located in the first transmembrane domain of the transporter. Several in vitro tools predict the exchange of a highly conserved amino acid.

Conclusion: We postulate that this is the first described famliy of non-Arab origin with GLYT1 encephalopathy and the first family with a severe prenatal phenotype with increased nuchal translucency and arthrogryposis caused by a *missense* variant in *SLC6A9*.

U. Grasshoff: None. **S. Beck-Woedl:** None. **T. Haack:** None. **K. Kagan:** None. **S. Haen:** None. **E. Botzenhart:** None.

E-P10.09
A novel pathogenic *MYH3* mutation in a child with Sheldon-Hall syndrome and vertebral fusion

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Introduction: Sheldon–Hall syndrome (SHS), also named distal arthrogryposis (DA) 2B, is characterized by congenital non-progressive contractures. Causative mutations in four genes have been described: *MYH3*, *TNNI2*,*TPM2*, and *TNNT3*. We report the first patient with typical SHS phenotype and exceptional vertebral fusions.

Materials and Methods: An 8.5-year-old boy was evaluated for dysmorphic facial features (triangular face with prominent nasolabial folds and chin, arched eyebrows, and downslanting palpebral fissures), camptodactyly of both hands combined with clinodactyly of toes, and several tendon retractions. Left hand X-ray showed a complete bony lunotriquetral coalition. Spine MRI and CT scan revealed multiple cervical and dorsal vertebral fusions. Patient's karyotype and CGH-array were normal. On suspicion of SHS, all the coding exons of *MYH3* and of the adjacent intronic regions were investigated by high throughput next generation sequencing (NGS).

Results: The novel, very rare heterozygous variation c.859T>G, p.Phe287Val was identified. This mutation produces a substitution of a Phenylalanine with a Valine at the position 287 and was predicted to be pathogenic by all the prediction programs used.

Conclusions: *MYH3* mutations are responsible for *MYH3*-related disorders: DA1, DA2A, DA2B, DA8, and autosomal dominant spondylocarpotarsal synostosis (Table). Our patient's phenotype suggests the existence of a phenotypic continuum for these disorders and a pivotal role for *MYH3* in regulating muscular and bone morphogenesis.

Acronyms	Alias	OMIM #	Main clinical features	Responsible genes	Affected MYH3 domains
DA1	Digitotalar dysmorphism	108120	Camptodactyly and clubfoot, hypoplastic interphalangeal creases	*MYH3, TNNI2, TNNT3, TPM2*	Head
DA2A	Freeman-Sheldon syndrome	193700	Contractures of hands and feet, scoliosis, oropharyngeal abnormalities with distinctive "whistling-face"	*MYH3*	Head, coiled coil (IQ motif)

Table (continued)

Acronyms	Alias	OMIM #	Main clinical features	Responsible genes	Affected MYH3 domains
DA2B	Sheldon-Hall syndrome	601680	Distinctive face, calcaneovalgus deformity, camptodactyly, ulnar deviation	*MYH3, TNNI2, TNNT3, TPM2*	Head, neck-coiled coil
DA8	Autosomal dominant multiple pterygium syndrome	178110	Multiple pterygia, congenital contractures of limbs, severe scoliosis, vertebral fusions	*MYH3*	Head, tail
ADSS	Autosomal dominant spondylocarpotarsal synostosis	272460	Progressive vertebral fusions, carpal and tarsal coalitions, clinodactyly, short stature	*MYH3*	Head, coiled coil

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E-P10.10
A novel variation in a patient with Sjogren-Larsson syndrome

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Introduction: Sjogren-Larsson syndrome (SLS) is a rare autosomal recessive disorder with neurocutaneous symptoms. Mental retardation and spasticity are among major clinic symptoms followed by less frequently symptoms such as seizures, retinal abnormalities and photophobia. SLS is caused by mutations in the aldehyde dehydrogenase family 3 member A2 (ALDH3A2) gene that encodes a fatty aldehyde dehydrogenase (FALDH).

Material and Methods: In this case, six years old boy consulted to our clinic with complaints of ichthyosis and inability to walk. The patient is son of a consanguineous couple who are first cousins. Brain MRI had demonstrated bilateral periventricular subcortical white matter leukomalacia. Karyotype and ALDH3A2 gene analysis planned due to these results.

Results: Karyotype analysis showed no constitutional abnormality. Next generation sequencing analysis of ALDH3A2(NM_001031806.1) gene showed homozygous substitutionof c.799-1G>A(IVS5-1G>A) at the last nucleotide of the 4th intron. This variant, is analysed in silica databases of 'Mutation Taster' and 'Human Splicing Finder' and suggested to be pathogenic. Parental genotypes showed thatboth parents had the same mutation heterozygously.

Conclusion: Uptodate, more than 90 different mutations of ALDH3A2 in SLS patients published. Since the location of the variant we are presenting, is on the splice site, it is probable that the variant can cause misregulation of splicing and can have effects on mRNA or protein products of the gene or the regulatory elements binding introns. According to databases, this variant might affect the protein features because of the alteration within splice site, likely to disrupt normal splicing.

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E-P10.11
A novel frameshift variant in *TNPO3* - a report of the second LGMD1F family

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Introduction: Limb-girdle muscular dystrophy, type 1F (LGMD1F) is an ultra-rare autosomal dominant myopathy caused by heterozygous mutations in the *TNPO3* gene. The sole previously reported LGMD1F family manifests notable variation of the phenotype regarding the age of onset, presentation and progression of muscle weakness affecting pelvic and shoulder muscles. Owing to the wide variation in the clinical picture of LGMDs, the precise diagnosis of these disorders is challenging. With the implementation of massively parallel sequencing (MPS) assays, the diagnostic accuracy and clarification of the genetic background of these diseases has been significantly improved.

Material and Methods: We used a previously developed targeted gene panel, MYOcap, to screen for possible pathogenic variants in two affected members of a Swedish family with a childhood-onset muscle weakness affecting proximal as well as distal muscles. The MYOcap assay consists of 265 genes and 5394 exons that are known or suspected to cause muscular dystrophy or myopathy.

Results: Our MYOcap assay identified a novel heterozygous single nucleotide deletion c.2757delC in the affected members of the family. The deletion causes a frameshift in

exon 22 of the *TNPO3* gene and is predicted to result in extension of the reading frame by 20 codons downstream (p.R920GfsX20).

Conclusions: The predicted consequence of c.2757delC mutation is very similar to the previously reported mutation in the published LGMD1F family (c.2771delA p. *924Cext15). In the family reported here, the phenotype was more compatible with congenital myopathy with slow progression during adult and late life.

A. Väisänen: None. **O. Danielsson:** None. **S. Penttilä:** None. **A. Vihola:** None. **B. Udd:** None.

E-P11 Multiple Malformation/anomalies syndromes

E-P11.01
Distal 11q monosomy - clinical, endocrinological, molecular data for a patient

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Jacobsen syndrome (JS), a rare contiguous gene syndrome is due to partial 11q deletion. There is a wide severity spectrum of the clinical phenotype but typical features include growth and psychomotor retardation, facial dysmorphism and thrombocytopenia. The deletion size ranges from ~7 to 20Mb, with the proximal breakpoint subband 11q23.3 to telomeric 11q. The literature data shows that the severity of clinical features in patients with JS is not clearly correlated with deletion size. The deletion occurs *de novo* in 85% of reported cases. We have evaluated a 7 years 11 months old patient with thrombocytopenia, facial dysmorphism, failure to thrive, dwarfism, microcephaly, long flat philtrum with thin lips, prominent columella, psychomotor retardation. Familial anamnesis revealed only cataract for patient's father. The karyotype was normal. Array-CGH notice a 6 Mb deletion on 11q24-q25. The genetic tests for parents are in work. We identified only a level of IGF1 at the lower limit of normal, although the literature data indicated GH deficiency or primary hypothyroidism in patients with JS. Special attention should be focused on bleeding concerns related to platelet number. This patient's development will be observed multi-disciplinary to review the progress.

E. Braha: None. **I. Gherlan:** None. **V. Plaiasu:** None. **O. Popa:** None. **E. Gorduza:** None.

E-P11.04
Report of a child with a nearly complete 17p duplication

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Introduction: The phenotypic characteristics of the duplications are variable according to their size and the gene regions they contain. Such chromosomal gains that generate suspicious images at chromosome analysis can be fully demonstrated by the array CGH method.**Materials and Method:** Firstly chromosome analysis was performed and a chromosomal change was found. To investigate the basis of the change, chromosome analysis in the parent and array CGH for the patient were performed.

Results: A 2.5-month-old girl was referred to our clinic because of the growth retardation and presence of triangular face, high palate, and low ear. Patent Foramen Ovale, secundum ASD and right cerebellomedullary cervical cyst, myelination delay, slit ventriculs and corpus callosum partial agenesis were present in the brain MRI of the SGA baby with hypotonia, feeding difficulty, apnea, GER. The chromosome analysis of the patient was found as 46,XX,der (15)add(15)(p13) subsequently to clarify the infrastructure of this change, parents' chromosome analysis and patient's array CGH were performed. Mother was detected as 46,XX, t(15; 17)(p13p11.2). In array CGH, the patient was found to have 3 copies in the short arm of chromosome 17 at the nucleotides between chr17: 525-22,084,950 in size 22084.425 kbp, 17p13.3p11.2.

Conclusions: Although balanced translocation result in normal phenotype in carriers, it is important that these changes could cause both balanced and unbalanced changes in their children. Evaluation of the array CGH method together with chromosome analysis is an effective method for detecting such uncommon chromosomal anomalies so that unbalanced abnormalities can be fully demonstrated.

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E-P11.05
Prenatal analysis of hypoplastic left heart Syndrome associated with a 22q11 microdeletion

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The 22q11 deletion syndrome is one of the most common microdeletions associated with a broad spectrum of abnormalities. The fetal phenotype associated with a 22q11 deletion is poorly described in literature but genetic analysis

is usually indicated when a congenital conotruncal heart defect is detected. Hypoplastic left heart syndrome associated with a 22q11 deletion syndrome is very rarely reported, while mutations in the GJA1- and the NKX2gene are known to be associated with this heart malformation. Here we report on a 35 year old pregnant woman who was referred to our institute for prenatal cytogenetic analysis because ultrasound examination showed a hypoplastic left heart syndrome with an atresia of the aorta and the mitral valves. Conventional karyotyping revealed an apparently normal female karyotype. After genetic counselling, array-CGH was performed and revealed a deletion of 1,3 Mb in the critical region of the 22q11 deletion syndrome. This deletion was confirmed by FISH. Cytogenetic analysis of the patient and the childfather must be performed to reveal if the deletion is familial or de novo.This case clearly illustrates the importance of performing prenatal Array-CGH in cases of severe heart malformations, and shows that genetic analysis for a 22q11 deletion might be also useful in cases of hypoplastic left heart Syndrome.

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E-P11.07

De novo interstitial deletion of 2q22.3-24.1

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We report on a nineteen months old boy with developmental delay, epilepsy and hydronephrosis. Birth-weight, length and head circumference were below 3rdcentile. After birth, hypotonia, feeding difficulties and a neonatal seizure occured. At presentation he crawled without any speech development. Length and head circumference were below 3rdcentile, weight 75th-90th centile. He showed narrow and short palpebral fissures, an epicanthus, long eyelashes, small nose, broad nasal bridge, low-set ears with large lobes, open-mouth with tented upper lip, small chin, small hands and feet with 2/3-syndaktyly. Array CGH analysis uncovered an interstitial 6.5 Mb deletion of 2q22.3-q24.1 confirmed in the child and excluded in parents by FISH analyses. In literature the 2q23.1 deletion syndrome is characterized by intellectual disability, speech-impairment, autistic-like behavior, sleep abnormalities, seizures, ataxia, microcephaly, hypotonia, initially feeding difficulties, later hyperphagia, coarse face, abnormalities of the ears and the eyes, open mouth, prominent incisors, tented upper lip, short hands and feet. The size of the described deletions varies (38 kb to 19 Mb) with the smallest region of overlap contains only the MBD5-gene. The protein encoded by MBD5 is expressed in brain and is involved in transcriptional regulation interacting with e.g. MECP2. Symptoms of isolated MBD5 alterations and 2q23.1 deletions overlap but some features (e.g. microcephaly, hyperphagia, open mouth with downturned corners, small hands/feet) are observed predominantly in 2q23.1 deletion, suggesting further deleted genes contribute to an extended phenotype. Our case suggests that a recognizable phenotype and facial gestalt can be defined in patients with a 2q23.1 deletion.

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E-P11.08

A *de novo* 8q22.2q22.3 interstitial microdeletion in a girl with developmental delay and congenital defects

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Interstitial deletions of the long arm of chromosome 8 are rare. We present a 3.5-year-old girl with developmental delay and congenital abnormalities due to *de novo* 8q22.2q22.3 microdeletion. The girl was born to healthy non-consanguineous parents at full term. Fetal ultrasound showed mild growth retardation. Birth weight was 2670 g, height 50 cm, OFC 31 cm. The patient had congenital hip dysplasia, bilateral foot deformity, and radioulnar synostosis. Dysmorphic features included up-slanting palpebral fissures, wide eyebrows, altered dermatoglyphics, short halluces, and hypertrichosis on the back, arms and forehead. Echocardiogram showed open *foramen ovale* and small secondary atrial septal defect. The psychomotor and language development was severely delayed. She had stereotypic movements, including hand waving and head twisting. The patient had a seizure episode at age 2.5 years, but EEG showed no epileptic activity. The girl had hypermetropia and atopic dermatitis. 8q22.2q22.3 deletion, 4.9 Mb in size (arr[hg19] 8q22.2q22.3 (99,938,470-104,936,546)x1) was identified by SNP-array analysis. *De novo* origin of the deletion was confirmed by RT-PCR as both parents are not the carriers of the deletion. Our patient shares the majority of the features described in previously reported seven patients with overlapping deletions, including radioulnar synostosis, previously described in one of these patients. We conclude, that the 8q22.2q22.3 microdeletion results in a recognizable phenotype with specific facial features and congenital abnormalities. More patients have to be

evaluated to establish a phenotype-genotype correlation and variability of clinical features. This research was funded by a grant (No. S-MIP-17-19/LSS-150000-1179) from the Research Council of Lithuania.

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E-P11.09

AHDC1 gene truncating 1p36.11p35.3 microdeletion in a patient with developmental delay, dysmorphic features and congenital heart defects

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Xia-Gibbs syndrome is a recently described genetic syndrome due to heterozygous truncating mutations in *AHDC1* gene on chromosome 1p36.11. The syndrome is characterized by global developmental delay, hypotonia, obstructive sleep apnea, intellectual disability and seizures. Congenital heart defects were not described in patients with Xia-Gibbs syndrome. The patient was a 1,5-years-old girl, the first child of healthy non-consanguineous parents, born from the fifth pregnancy (there were four miscarriages) complicated by polyhydramnios with small birth weight (3‰). Pulmonary artery stenosis with atrial septal defect, transient congenital stridor with laryngeal hypokinesis, corpus callosum hypoplasia with ventriculomegaly were diagnosed in the neonatal period. Feeding difficulties with gastroesophageal reflux and failure to thrive, strabismus divergens with hypermetropia, muscular hypotonia, psychomotor retardation and dysmorphic features were evident from the first months of age. Whole-genome SNP array analysis revealed a 1.1 Mb deletion in the 1p36.11p35.3 region, arr[GRCh37]1p36.11p35.3(27862451_29004746) x1.The mother's SNP array revealed no abnormalities, while the father was not available for testing. The deleted region involves 22 OMIM genes including one OMIM morbid gene, *AHDC1*, with the distal breakpoint of the deletion disrupting *AHDC1* introns 5-6 in the 3' untranslated region. Other genes with presumable haploinsufficiency (HI< 20; *FGR, RCC1, PPP1R8, MED18, EYA3, TAF12, DNAJC8*) do not have described functions in heart development except from *TRNAU1AP*, a transfer RNA selenocysteine 1 associated protein 1, that has an essential role in the synthesis of selenoproteins. In conclusion, presented patient exhibits typical clinical features of Xia-Gibbs syndrome with additional cardiac phenotype presumably due to haploinsufficiency of *TRNAU1AP* gene.

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E-P11.10

A 3-year-old girl with Aicardi syndrome phenotype and _MECP2_ gene duplication

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Introduction: Aicardi syndrome (AIS) is a rare disorder characterized by agenesis of the corpus callosum (ACC), distinctive chorioretinal lacunae and infantile spasms. It occurs almost exclusively in females. The cause is probably a *de novo* dominant X-linked mutation, lethal in 46,XY males. Here we report a case of a 3-year-old girl with typical AIS phenotype and *MECP2* duplication.

Results: Our patient, a 3-year-old girl, has moderate global developmental delay. In infancy she developed infantile spasms and later on myoclonic seizures. She has axial hypotonia and wide gait with a pronounced spastic pattern. Brain MRI revealed ACC with additional anomalies. Ophthalmologic exam showed two chorioretinal lacunae in the left eye. She is prone to viral infections and has mild facial dysmorphia. Chromosomal microarray revealed 254 kb long *de novo* Xq28 duplication, which encompasses six genes, including *MECP2* and *IRAK1*.

Conclusion: In males, *MECP2* duplication features are infantile hypotonia, developmental delay/intellectual disability, poor/absent speech, progressive spasticity, recurrent infections and seizures. Most female carriers are asymptomatic due to skewed X-inactivation. Only 19 symptomatic females have been reported so far. Our patient's phenotype is consistent with *MECP2* duplication, except chorioretinal lacunae that are a hallmark of the AIS. This is the first case linking these two conditions, pointing to the possibility that the gene for AIS might be within the Xq28 region. The gene of interest is *FLNA*, located distally of the duplication in our patient. *FLNA* causes periventricular heterotopia often seen in AIS cases which makes him a good candidate for AIS phenotype.

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E-P11.11

Highly frequent mutations in a Spanish Alström cohort

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Alström syndrome (ALMS) is a rare disease whose symptoms include defects of cardiac function, progressive loss of vision, early onset diabetes mellitus type 2, obesity, deafness, growth retardation and renal failure. It is caused by mutations in the *ALMS1* gene which encodes the ALMS1 protein, a structural component of centrosome involved in intracellular transport among many other roles.

We analyzed the sequence of *ALMS1* in a cohort of 11 unrelated ALMS families. Several mutations were identified in exons 7, 8, 16 and 17. Most of the mutations were found in exon 8. Two mutations had a high incidence in the cohort: p.(Tyr1714 *) (exon 8; rs772136379) with 36% and p.(Ser3872Tyrfs * 19) (exon 17), with 27%. We described one homozygote for p.(Tyr1714 *) mutation and one for p.(Ser3872Tyrfs * 19), all the other families with this mutations were compound heterozygotes. Segregation analysis showed that the p.(Tyr1714 *) mutation is linked to the SNP rs45608038 which presents a low frequency (≈2%) in European populations.

ALMS patients usually account for private mutations. For the first time we can see mutations with a large incidence in an ALMS cohort. Additional analyzes are underway to decipher whether these families maintain a genealogical relationship, although families have been recruited from different national geographic areas and none of them reported to have kinship relations with the other ones. If the common heritage of mutation p.(Tyr1714 *) were demonstrated, we could be talking about a founder allele for Spanish patients.

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E-P11.12
Amelogenesis imperfecta as a pathognomonic oral finding in enamel renal syndrome

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Introduction: Enamel renal syndrome is an autosomal recessive disorder. Amelogenesis imperfecta and gingival enlargement, as oral findings, in addition to nephrocalcinosis, are the diagnostic clues for the syndrome. In this research, we present the clinical and oral findings in two patients with enamel renal syndrome.

Material and Methods: The two patients were complaining primarily of amelogenesis imperfecta and gingival enlargement. A gingival biopsy was taken, Abdomen ultrasound was requested, and molecular studies were performed.

Results: Gingival biopsy revealed fibro-osseous lesions, renal calculi were found by ultrasonography, and Fam20A mutation was detected. The collective findings confirmed the enamel renal syndrome.

Conclusion: Amelogenesis imperfecta as a dental anomaly could be a powerful diagnostic evidence to certain rare diseases. Dentists should be aware of dental anomalies associated with different syndromes.

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E-P11.14
Detection of Chromosomal Imbalances in an Argentinean group of patients with Intellectual Disability or Multiple Congenital Anomalies

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Introduction: ArrayCGH is a significantly high resolution method to scan the genome for gains and losses of chromosomal material. Although in several countries it is a first-tier test for patients with intellectual disability (ID) or with multiple congenital anomalies (MCA), in our country it represents an expensive technique and a very few laboratories have begun to implement it. Karyotyping remains to be the routine study in public health.

Aim: To report the findings in an Argentinean cohort of patients with ID and patients with MCA.

Materials and Methods: we studied 165 patients with two arrayCGH plataforms (Agilent ISCA-v2-60K and KaryoArray®v3.0-8x60K): 125 had ID and 40 had MCA. They did not show a cytogenetic anomaly or had a failed karyotype test.

Results: We found that 14/125 (11%) ID patients had causal or potentially causal CNVs: 11 were recognizable syndromes and 2 had an imbalance associated with a microdeletion syndrome accompanied with other pathogenic imbalance. Besides, 10/40 (26%) MCA patients presented causal or potentially causal CNVs: 2 were chromosomal anomalies, 3 were recognizable syndromes and 1 had a microdeletion syndrome accompanied with other pathogenic imbalance.

Conclusion: ArrayCGH was useful to perform an accurate analysis of microdeletions or duplications that could not be detected by standard karyotyping. The percentage of pathogenic imbalances was higher in patients with MCA than in those with ID in accordance with the data reported in the literature.

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E-P11.15
A case of MAGEL2 mutation in a patient with prenatally diagnosed distal arthrogryposis and severe neonatal hypotonia

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Here we present a case of 6-months-old girl with prenatal signs of distal arthrogryposis, severe neonatal hypotonia and de novo mutation in the MAGEL2 gene. She was born after first pregnancy of non-consanguineous parents. Decreased foetal movements and anomaly in fingers flexion without polyhydramnios were noticed prenatally. The foetal karyotype and 8x60 K Agilent array CGH were normal. The birth was at term with weight and high on the 75th percentile. Severe axial hypotonia and flexed 3rd, 4th and 5th fingers were noticed. There were feeding difficulties and vesicoureteral reflux with moderate renal insufficiency. The dysmorphology examination showed bilateral single palmar crease, frontal bossing and prominent ears. The panel of arthrogryposis multiplex congenita genes detected a de novo heterozygous frameshift mutation in the MAGEL2 gene (15q11.2), c.1996dup, p.(Gln666Prfs*47). This gene is described implicated in Shaaf-Yang syndrome with a Prader Willi-like phenotype. MAGEL2 is maternally imprinted gene and mutation occurred on the paternal allele. With the presented case we would like to highlight the importance that MAGEL2 is included in the panel of genes tested in patients presenting a neonatal hypotonia with or without arthrogryposis or polyhydramnios.

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E-P11.16
A novel *ECEL1* mutation expands the phenotype of distal arthrogryposis multiplex congenita type 5D to include pretibial vertical skin creases

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Background: Arthrogryposis multiplex congenita (AMC) is a heterogeneous disorder characterized by multiple joint contractures often in association with other congenital abnormalities. Pretibial linear vertical creases are a rare finding associated with arthrogryposis, and the etiology of the specific condition is unknown.

Materials and Methods: Whole exome sequencing, segregation analysis and bioinformatics analysis were used to genetically characterize a boy from a consanguineous family, presenting with AMC and pretibial vertical linear creases on the shins.

Results: Whole exome sequencing and variant analysis revealed homozygous novel missense variants of *ECEL1* (c.1163T>C, p.Leu388Pro, NM_004826), and *MUSK* (c.2572C>T, p.Arg858Cys, NM_005592). Both variants are predicted to have deleterious effects on the protein function, with amino acid positions highly conserved among species. The variants segregated in the family, with healthy mother, father and sister being heterozygous carriers and the index patient being homozygous for both mutations.

Conclusion: We report on a unique patient with a novel *ECEL1* homozygous mutation, expanding the phenotypic spectrum of Distal Arthrogryposis Multiplex Congenita Type 5D to include vertical linear skin creases. The homozygous mutation in *MUSK* is of unknown clinical significance. *MUSK* mutations have previously shown to cause Congenital myasthenic syndrome, a neuromuscular disorder with defects in the neuromuscular junction.

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E-P11.17

Baraitser-Winter Syndome: A Turkish boy with a novel *ACTB* variant

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Introduction: Baraitser-Winter syndrome is a rare developmental disorder characterized by hypertelorism, ptosis, ridged metopic suture, high-arched eyebrows and brain malformations. Other common manifestations include hearing loss, short stature, seizures, intellectual deficit of variable severity, and abnormalities of the urinary system. This syndrome is caused by missense variations in *ACTB* or *ACTG1*.

Materials and Methods: We diagnosed a 5-year-old Turkish boy as Baraitser Winter syndrome with typical clinical features. All coding exons and the flanking intronic regions of the *ACTB* and *ACTG1* genes were amplified by PCR followed by bidirectional direct sequencing. Variations were detected by comparing the reference sequences and interpreted by *in silico* predictive programs.

Results: We identified a novel heterozygous sequence change, c.332A>G [p.(Asn111Ser)] which is located at the exon 3 of *ACTB*. This variation is not present in single nucleotide polymorphism and 1000 genomes databases; moreover, *in silico* analysis using SIFT, PolyPhen2 and Mutation Taster predicts as potentially deleterious. Parental carrier testings confirmed that these variants occured as *de novo* events which confirmed a *de novo* event.

Conclusions: The aim of this paper is to highlight the importance of the genotype-phenotype variability that needs to be considered during clinical assessment. Fuctional studies and additional case reports are required to understand the affect of the mutation and genotype-phenotype correlation.

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E-P11.18

Duplications in the 11p15 imprinted region: report of three patients with Beckwith-Wiedemann syndrome

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Introduction: Beckwith-Wiedemann syndrome (BWS) is characterized by overgrowth, macroglossia, abdominal wall defects, and a high risk of childhood tumors. BWS is caused by various 11p15 genetic or epigenetic defects leading to abnormal expression of imprinted genes. The genes in the 11p15 region are organized into two imprinted domains: ICR1 and ICR2. A small number of BWS cases (2-8%) are due to copy number variations (CNVs) in the chromosome 11p15.

Materials and Methods: We present epigenetic/genetic/clinical profiles of three patients with BWS. Molecular analyses were performed on leukocyte DNA and comprised: MS-MLPA, SNuPE, arrayCGH and FISH.

Results: In all patients aberrant methylation was associated with the presence of paternal origin CNVs, including: duplication of the whole 11p15 region (unbalanced translocation with involvement of 7p22.3 deletion), duplication of ICR1 and part of ICR2, and duplication of ICR1. Patients demonstrated typical features for BWS, additionally, the patient with duplication of the whole 11p15 region had developmental delay and facial dysmorphism. All identified CNVs were present on maternal alleles of the patients' healthy fathers.

Conclusions: The study shows that interpreting of 11p15 CNVs is challenging and phenotypic consequences may depend on the localization, size, as well as the parental origin of the aberrant segment. A complex molecular approach to analyze 11p15 region is required for accurate diagnosis of BWS allowing the recurrence risk determination and proper genetic counseling.

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E-P11.20

A female with a maternally inherited nonsense mutation in PHF6 with expanded phenotypes of Borjeson-Forssman-Lehmann syndrome

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Introduction: Borjeson-Forssman-Lehmann syndrome (BFLS) is a rare X-linked disease caused by *PHF6* variations. Patients with BFLS present moderate to severe intellectual disability, developmental disorders, obesity, epilepsy, hypogonadism, characteristic faces and anomalies of fingers and toes. However, the genetic findings and clinical observations are exclusively based on patients of European ancestry. Phenotypic spectrum in other populations is not well delineated.

Materials and Methods: Whole-exome sequencing was conducted in a female Chinese patient with major features of BFLS. Sanger sequencing was performed to validate the mutation and inheritance. The pattern of X-chromosome inactivation was evaluated by assays of differential methylation in the genes between the active and the inactive chromosome X based on Methylation-specific PCR. Phenotypic comparison was conducted based on this patient and cases in literature.

Results: A novel nonsense heterozygous mutation of c.673C>T; p.R225X was identified in the patient, which is inherited from her unaffected mother. Both the patient and her mother showed highly skewed X-inactivation. This patient showed complete deficiency of growth hormone, which was not reported before, and adverse effect emerged after treatment of growth hormone. She also exhibited phenotypes partially overlapping with Coffin-Siris syndrome.

Conclusions: Complete deficiency of growth hormone is a new phenotype presented in our Chinese patient with BFLS. X-inactivation pattern in the peripheral blood may not be a reliable predictor of the BFLS presentation. (This study was supported by Grant No. 81500972, to YF; No. 81670812, to YGY.)

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E-P11.21

A Novel Nonsense Mutation In The *EYA1* Gene Found In A Patient With BOR Syndrome

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Introduction: Branchio-oto-renal syndrome (BOR), is an autosomal dominant disorder characterized by the coexistence anomalies of the external,middle or inner ear malformations with pre-auricular pits or tags, conductive, sensorineural or mixed hearing loss, branchial cleft anomalies, and renal anomalies ranging from mild, asymptomatic hypoplasia to complete renal agenesis.

Clinical Report: A 38 years old male patient was referred us because of chronic renal insufficiency and hearing impairment. Clinical examination showed auricular deformity, pre/post-auricular pits, operated bilateral branchial fistulae and unilateral peripheral facial nerve palsy. Abdominal ultrasound revealed unilateral kidney hypoplasia. An audiogram showed right sided moderate mix hearing loss and left sided sensorineural hearing loss. Laboratory tests resulted in high blood urea nitrogen (BUN) 23 mg/dL and serum creatinine 1.81 mg/dL; low glomerular filtration rate (GFR) 46 mL/min. We performed sequence analysis for the *EYA1* gene which is responsible for 40% of the etiology.

Results: We found a premature stop codon mutation on p. Tyr182* (c.546C>G) of the *EYA1* gene. It is a novel mutation and based on ACMG 2015 criteria, it was considered as a pathogenic variant that clearly causes severe damage on protein function.

Conclusion: We present this case of BOR syndrome with a novel mutation at *EYA1* gene in order to contribute to the literatüre. Reference Richards S, Aziz N, Bale S *et al*, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*2015;**17**:405-424.

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E-P11.22

New case of S. SIfrim-Hitz-Weiss (#617159) caused by de novo mutation in CHD4 gene

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Introduction: Sifrim-Hitz-Weiss syndrome is an autosomal dominant intellectual disability syndrome recently

described, associated with variable congenital defects, including cardiac, skeletal, and urogenital ones. Some patients may have short stature, macrocephaly, hearing loss, and dysmorphic facial features. It is caused by mutation in CHD4, an ATP-dependent chromatin remodeler gene.

Material and Methods: We described the case of a 24 year-old-man born to healthy non-consanguineous parents. Pregnancy and neonatal period were normal. He presented with psychomotor retardation, hypotonia and muscle weakness with normal electromyogram. He was schooling with support and speech therapy. After finishing the compulsory schooling he studied to be a social worker, and nowadays he is working. At the age of 11 scoliosis was detected, he needed corset during 3 years. 9 years later, bilateral cryptorchidism was diagnosed after an ultrasound performed because of abdominal pain. He underwent surgery of testes, histological analysis didn`t show malignancy. He is followed up by an Endocrinologist due to the diagnosis of hypogonadotropism- hipergonadotropic and subclinic hypotiroidism. His stature is normal (p13) and he has dismorphic features: square-shaped face, relative macrocephaly, hypertelorism, bilateral ptosis and small low set ears. After ruling out cromosomopathy, de novo mutation c.T4012G was detected by exome sequencing.

Conclusions: We present a new case with some of the characteristic features: hearing loss, hypogonadism, delay neurodevelopment, muscle weakness, dysmorphic facial features; but without cardiac anomalies nor intelectual disability, showing the variable expressivity in this syndrome. It should be included in the diferential diagnosis of rasopathies, facial dysostosis and Charge Syndrome.

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E-P11.23

Cytogenetically pseudobalanced chromosome 2 structural rearrangements associated with 2q23.1 microdeletion syndrome

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Introduction: 2q23.1 microdeletion syndrome ("pseudo-Angelman" syndrome) is characterized by speech delay and intellectual disability, autistic features, inappropriate laughter and short stature. The disease is mainly associated with MBD5 loss. Over 100 cases of the syndrome are described in the available literature. Here, we report on two cases of cytogenetically pseudobalanced chromosome 2 structural rearrangements associated with 2q23.1 microdeletion syndrome.

Materials and Methods: Using karyotyping and SNP array (Affymetrix Cytoscan HD), we detected two cases of 2q23.1 microdeletion resulted from a paracentric inversion and reciprocal translocation in a cohort of 466 patients with intellectual disability, autistic features and congenital malformations.

Results: The patients were a 2-year-old girl and a 6-year-old boy, exhibiting 2q22.3q24.1(148,513,845-155,196,310)×1, 46,XX,der(2)inv(q21.1q22.3)del(q22q24) and 2q23.1q23.3(148,851,963-151,316,465)×1, 2q22.2q24.1(143,753,727-155,408,790)×1~2, 46,XY,t(2;11)(q31.1;q21), respectively. Common clinical features were motor, speech and developmental delay, gait disturbance, myopia. Additionally, the girl exhibited microcephaly, cleft palate, muscle hypotonia, hearing loss, congenital heart disease. The boy presented with seizures, protruding ears, micrognathia, joints hypermobility. Beside *MBD5* deletion, both cases demonstrated a loss of *ACVR2A, ORC4, EPC2, KIF5C, LYPD6, MMADHC, RND3, NMI, TNFAIP6, RIF1, NEB, ARL5A, CACNB4, STAM2, PRPF40A, RPRM, GALNT13, FMNL2* and *ARL6IP6*.

Conclusions: Rare cases of pseudobalanced chromosomal rearrangements exemplify the intrinsic need for genome-wide CNV scan in molecular cytogenetic diagnosis. Furthermore, these cases demonstrate the complexity of pseudobalanced chromosomal abnormalities that can be easily overlooked. Supported by RSF (14-15-00411).

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E-P11.24

Intrafamiliar clinical variability of Circumferential Skin Creases Kunze Type caused by a novel heterozygous mutation of N-terminal *TUBB* gene

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Circumferential skin creases Kunze type' (CSC-KT; OMIM 156610, 616734) is a rare disorder characterized by folding of excess skin, which leads to ringed creases, primarily of the limbs, known as "Michelin Tyre Baby Syndrome" (MTBS). Additionally, CSC-KT patients exhibit facial dysmorphism (epicanthal folds, upslanting palpebral fissures, hypertelorism), cleft palate, growth retardation, intellectual disability (ID) and multiple congenital malformation. Recently, 2 heterozygous mutations in *TUBB* gene and 4 mutations (both homozygous and heterozygous) in *MAPRE2* gene were identified in 3 and 4 patients, respectively, with CSC-KT. In the 3 *TUBB* gene-related CSC-KT patients, all mutations falled in the N-terminal domain of the gene and were *de novo*. Mutations in the C-terminal of *TUBB* gene are associated to microcephaly and structural brain malformation, in the absence of sign or symptoms of the CSC-KT. Here, we report a 9-year-old boy in whom the diagnosis of CSC-KT was suggested, based on the presence of MTBS, typical facial dysmorphism, microcephaly, severe ID and brain malformation, including cortical atrophy and corpus callosum hypoplasia. Sanger sequencing identified a novel heterozygous c. 218 T>C (p. Met73Thr) variant in the N-terminal of *TUBB* gene. The variant was inherited from the mother that was retrospectively evaluated, and showed MTBS with a spontaneous improvement and no other signs of CSC-KT. We report the first transmitted TUBB gene-related CSC-KT caused by a novel heterozygous mutation of N-terminal of the gene. These data further validates the role of TUBB mutations in causation of this condition and definitely includes the CSC-KT syndrome in tubulinopathies.

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E-P11.25

Clinical exome sequencing in genetic diseases - interpretation of family trio

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Introduction: Clinical analysis of exome is method for comprehensive diagnosis and interpretation undiagnosed genetic disease and rare disease. Diagnoses are based on known or presumed pathogenic variants in genes already associated with a similar phenotype. Here, we extend this paradigm by evaluating novel bioinformatics approaches to aim? identification of new gene-disease associations.

Materials and Methods: We performed massive parallel sequencing on MiSeq (Illumina) using panel Clinical Exome kit by Sophia Genetics, followed by Sanger sequencing, CNV and MLPA analysis. This solution consists of 116,355 individually designed probes that span approx.. 11 Mb of target regions covering more than 4,900 genes, with known mendelian/inherited disease-causing mutations.

Results: Altogether, we have tested 5 family trios (mother, father and child) using Clinical Exome kit. During the testing of clinical exome we required to obtained all the relevant genetic diagnosis and clinical phenotype information of the analysed patients. These 3 families were diagnosed mainly of the heritable disorders like Ollier syndrome···. In summary, we have found causal DNA variants in genes already associated with a phenotype. We have also identified pathogenic or potential pathogenic variants in other genes, which are probably not associated with disease, however their clinical interpretation is unclear and should be finalized in the cooperation of clinical and molecular geneticist. These results demonstrate the efficiency of exome sequencing approach in performing molecular diagnosis of undiagnosed genetic disease.

Conclusions: We have tested patients to detect a mutation in gene of undiagnosed genetic disease.

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E-P11.26

De novo apparently balanced complex chromosomal rearrangement involving three chromosomes with cryptic genomic imbalances

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Introduction: Complex chromosome rearrangements (CCR) are extremely rare in humans. Most apparently balanced CCRs are de novo and usually detected in phenotypically normal subjects. But in some cases found in patients with congenital abnormalities and/or mental retardation which may be due to cryptic genomic imbalance. We report an exceptional CCR in a child with mental retardation, microcephaly, muscular hypotension, corpus callosum hypoplasia.

Materials and Methods: G-banding analysis revealed a de novo CCR involving the chromosomes 4, 5, 7. By FISH

using different types of probes (PCP, MCB, subtelomeric) it was possible to clarify how the chromosomes were rearranged and to determine the breakpoints. aCGH was applied for detection of genomic imbalance.

Results: Conventional karyotyping showed translocation t(4;5;7)(q31;q32;p21)dn. MCB analysis of chromosomes 4, 5, 7 was employed to delineate the breakpoints in every chromosome. Unexpectedly we detected that a segment of 4 (q31.23q32.2) was inserted in the derivative chromosome 5 (q31.1). Arm-specific and subtelomeric probes were applied to confirm the insertion 4q in 5q. Furthermore, aCGH-analysis showed four cryptic deletions associated with the breakpoints on chromosomes 4 and 5.

Conclusions: Initially, CCR described here was interpreted as balanced, and derived from a three-way translocation between chromosomes 4, 5, 7. However, FISH and aCGH-analysis revealed that the rearrangement was far more complex than originally estimated involving a larger number of breaks. Only a combination of several different approaches was sufficient to resolve the nature of this CCR which had an unexpected level of complexity, involving 3 chromosomes, 5 breakpoints, 1 insertion and 4 deletions.

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E-P11.27
Detection of Complex Genetic Anomalies with Next Generation Sequencing

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Introduction: Next Generation Sequencing can be used to diagnose complex genetic changes in which conventional genetic methods are inadequate.

Materials and Method: Whole exome sequencing was done because the patient had multiple anomalies.

Findings-Results: A 17-year-old male with multiple anomalies was referred to our polyclinic for genetic evaluation and clarification of the diagnosis. There were chronic cough, torticollis, short stature, glaucoma and microphthalmia on the anamnesis and physical examination of the patient. Computerized spiral tomography of chest and magnetic resonance imaging of brain performed respectively, there were widespread bronchiectasis, multiple arachnoid cysts, ventriculomegaly and microphthalmia. 6 cousins and 2 siblings of the patient died due to similar findings and because his parents were relatives blood was taken from the family to perform whole exom sequencing. Homozygous mutation in the PRESS56 gene (c.571G>T), homozygous mutation in the MCIDAS gene (c.904C>T) were detected in the patient. Mother and father were found to be carriers in terms of these mutations.

Conclusions: Next generation sequencing should be preferred in patients with complex genotype resulting multiple anomalies.

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E-P11.28
Cytogenomic characterization of a complex small supernumerary marker chromosome leading to partial 4q and 21q duplications: clinical implication and review of the literature

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Complex small marker chromosomes (sSMC) consist of chromosomal material derived from more than one chromosome. Complex marker chromosomes derived from chromosome 4 and 21 are rare with only five cases described in the literature, and two listed in the online database of sSMCs (http://ssmc-tl.com/sSMC.html). We describe a patient who presents a complex sSMC derived from a maternal translocation between chromosomes 4 and 21, revealed by G-banding, MLPA, and array techniques. The final cytogenomic result was given as 47,XX, +der(21)t(4;21)(q32.1;q21.2)mat .arr 4q32.1q35.2 (158907036_190957460)×3, 21q11.2q21.2 (15016486_25605895)×3. There are 234 RefSeq genes in the 4q32.1q35.2 duplicated region, 74 of them are OMIM genes with 17 associated with diseases. Regarding the region in which there are no probes on SNP array, the duplicated region on chromosome 21 has 25.6 Mb from 21pter to 21q21.2 (1-25605895) and contains 121 RefSeq genes, 18 of them are OMIM genes and 2 are disease-causing genes. Our patient presents prominent forehead, flat face, upslanting palpebral fissures, hypertelorism, large nose with a prominent nasal bridge, downturned corners of the mouth, short neck, scoliosis, high arched palate, clinodactyly, tapered fingers and single palmar creases. Apart from the breakpoints, our patient shows a similar phenotype to the 4/21 sSMC cases and a compilation of the findings found in isolated 4q and 21q duplications. Neuropsychomotor developmental delay and facial dysmorphism were the most important findings. The wide range of phenotypes associated with sSMCs emphasizes the importance of detailed cytogenomic analyses for an accurate diagnosis, prognosis, and genetic counseling.

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E-P11.29

Possible involvement of FREM2 homozygous mutation in a Japanese family with unilateral small kidney determined by whole-exome sequencing

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Congenital anomalies of the kidney and urinary tract (CAKUT) constitute a major cause of chronic disease in humans. More than 50 genes have been reported as mutated in CAKUT affected cases. Recently, mutations in genes encoding Fraser syndrome protein 1 (*FRAS1*), FRAS1 related extracellular matrix protein 2 (*FREM2*) and glutamate receptor interacting protein 1 (*GRIP1*) cause CAKUT. A patient with consanguineous parents underwent whole-exome sequencing to identify the causative gene(s) for his unilateral small kidney, relatively hypouricemia and mental retardation. After WES, we narrowed down our search for candidate genes to 48 responsible for the small kidney phenotype. Lastly, we identified a *FREM2* homozygous mutation (c.C4030T, p.R1344C) and a *GRIP1* homozygous mutation (c.C692T, p.A231V) probably associated with unilateral small kidney. *In silico* analysis revealed that R1344C in FREM2 was probably damaging, and that A231V in GRIP1 was benign. R1344 in FREM2 is evolutionarily conserved, and is located within chondroitin sulfate proteoglycan repeats, which could be the binding domain for several growth factors. Furthermore, the late patient's brother and sister manifested a relatively bilateral small kidney and normal kidney. However, his genotyping was a *GRIP1* homozygous mutation without *FREM2* mutation, and her genotyping was a *FREM2* and *GRIP1* heterozygous mutation. Therefore, these results suggest that the mutation in *FREM2* is pathogenic and might be associated with the unilateral small kidney phenotype. This is the first report of homozygous missense mutation in *FREM2* found in a patient with a unilateral small kidney in a Japanese family.

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Phenotype variability of autosomal dominant congenital fibrosis of extraocular muscles CFEOM in the family with mutation in *TUBB3* gene

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Ophthalmoplegia and limited eye movement are frequently observed neurological abnormalities. It could be result of acquired diseases, inborn errors of metabolism or congenital defects. In some patients abnormalities in associated eyes structures often coexist with inborn defects of other organs being one of the symptoms of a specific syndrome, but sometimes it could be an isolated entity. One of them is congenital fibrosis of extraocular muscles (CFEOM). CFEOM is a complex condition with AR or AD inheritance and high clinical variability as a result of mutation in one of at least six genes. Some patients may have unilateral eye involvement. Here we report a family with CFEOM. In a 10-years-old boy ptosis of eyelids, exotropia and fingers camptodactyly were present since the neonatal period. Physical examination additionally revealed severe limited movement of eyes, small facial dysmorphic features. In other family members (mother, aunt, grandmother and cousin) only abnormalities in associated structures of eyes were reported. aCGH revealed in the boy a small 14q31.3q32.11 duplication of uncertain clinical significance. Based on clinical symptoms, a mutation in *TUBB3* gene has been suspected. A p.Arg190Cys (c.568C>T, rs267607162) mutation registered in the HGMD and in ClinVar as pathogenic variant was identified in affected boy and other family members. The CFEOM3A was confirmed in this family. Given high clinical variability in patients with CFEOM it is recommended to look for mutations in CFEOM genes in group of patients with congenital inborn defects and abnormalities in associated structures of eyes.

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Congenital heart defects in patient with new *KMT2D* frameshift mutation

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Introduction: Congenital heart defects (CHD) are the most common type of birth defect in newborns. CHD are also frequently accompanied by genetic syndromes. Usually, additional features along with CHD are observed in these syndromes. The genetic cause of the syndromes can be single-gene mutations and copy number variations. Because the clinical picture of patients with different syndromes can overlap, it is very important that a good clinical examination is performed, which leads to a better diagnosis of these conditions.

Material and Methods: A 10-moths old boy was referred for NGS analysis due to the CHD observed during the pregnancy. After birth vesicoureteral reflux, muscular hypotonia, cleft palate, and mild microcephaly was noted. The prenatal testing was performed for observed CHD, which included classical karyotyping, molecular karyotyping and QF-PCR for exclusion of aneuploides and microdeletions/microduplications syndromes. All genetic tests were negative. NGS analysis was performed with panel covering more than 4800 disease-associated genes. Analysis was initially focused on genes associated with CHD, but no clinically significant variants were detected. Only after the extended genetic analysis, which also included genes related to additional features observed in our patients, enabled us the detection of a previously undescribed frameshift mutation c.11093delG in *KMT2D* gene which is associated with Kabuki syndrome.

Conclusion: Broad spectrum of clinical features observed in Kabuki patients and its rare incidence disables the easy recognition of this syndrome. Our case presents the importance of a good clinical characterizations of the patients which undergo the NGS analysis for successful diagnostics.

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E-P11.32

Molecular diagnostics of Cornelia de Lange syndrome and related genetic disorders

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Introduction: Cornelia de Lange syndrome (CdLS) is a rare complex disorder with multiple structural and developmental defects characterized by typical facial features, intellectual disability, and congenital anomalies. It has been estimated to occur in about 1:10 000 individuals where severe forms of CdLS are revealed easilly, but as more mildly affected individuals have been reported, its actual prevalence may be much more common. Diagnostics of CdLS is complicated with a presence of clinically overlapping syndromes.

Methods: Our custom NGS panel (completed by MLPA) encompasses genes associated not only with a specific diagnosis (CdLS in this case) but also with diseases, which are hardly distinguishable from CdLS because of their phenotypic similarity.

Results: To date we have investigated 13 pacients with CdLS clinical diagnosis. Four of them (three children and one fetus) carried pathogenic variants in *NIPBL* gene or *SMC3* gene, so their diagnosis was confirmed on molecular level. Our custom NGS panel includes genes related with clinically overlapping syndromes, so we were able to identify pathogenic variants in *ANKRD11* gene (**KBG syndrome**) and *AFF4* gene (**CHOPS syndrome**) in two children patients.

Conclusions: For a routine molecular testing it's important to create custom NGS panels with a great prudence. Only in this case it can be a successful diagnostic tool which can solve the issue with molecular diagnostics of clinically overlapping syndromes.

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Widening the spectrum of congenital anomalies related to a partial 21q monosomy detected prenatally

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Introduction: A 29-year-old pregnant woman was referred at 20^{+4} weeks because of fetal anomalies. The couple was healthy, unrelated and of South American origin. Level II ultrasonography (US) revealed a cystic hygroma, complete agenesis of corpus callosum, aortic valvular stenosis with aortic hypoplasia, left ventricular hypoplasia and ventricular septal defect, flexion deformity of hands. Brain abnormality was confirmed at MRI.

Materials and Methods: Agilent Technologies 60K Chromosomal MicroArray (CMA) performed on amniotic fluid cells revealed a loss of 21,5 Mb of chromosome 21q11.2q22.12, encompassing many OMIM genes, 8 of which are known to be disease-associated. CMA also showed two small imbalances inherited from the healthy mother. The imbalance appeared as a complete chromosome 21 monosomy upon cytogenetic analysis of cultured cells. Maternal karyotype was normal, whereas paternal one revealed a 1p22-p36.1 paracentric inversion. FISH analysis with a probe specific for subtelomeric 21q regions (Vysis) was normal for both parents. The pregnancy was terminated. Fetal pathology and autoptic MRI confirmed all prenatal US findings while also identifying periventricular nodular subependymal heterotopia and very distinctive facial features (low-set and posteriorly set ears, frontal hirsutism with broad eyebrows, hypertelorism, a broad nasal bridge with anteverted nostrils, microretrognathia and high-arched palate).

Conclusions: This case, characterized by a broad loss of genetic material, widens the phenotypic spectrum of 21q deletions revealing a very peculiar prenatal picture. Similar but smaller imbalances have been described as definitely pathogenic in children with intellectual disability, hypoplasia of the corpus callosum, dysmorphic facial features and VSD.

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E-P11.34

Chromosomal microarray versus karyotyping for diagnosis of a rare inherited apparently balanced translocation involving chromosomes 13 & 21

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Introduction: Apparently balanced chromosomal rearrangement associated with an abnormal phenotype is a rare event, but with difficult management. In the past few years array CGH changed the diagnostic approach to many congenital diseases offering an accurate detection of chromosome imbalances.

Material and Methods: A 6 months old girl, the third child of the couple with history of infertility, was referred to our genetic center because of developmental delay, dysmorphic features, agenesis of corpus callosum, sacral dimple, abnormal skin pigmentation with linear intercostal localisation, growth retardation. Cytogenetic analysis showed a derivative chromosome 21 inherited apparently from her mother and arrayCGH result was: arr[hg19] 13q12.11-q12.3(20407295-31729705)x3,21q11.2-q21.3 (15485008-30013630)x1. FISH testing was necessary to clarify the results.

Discussion: The patient presented a visible abnormal chromosome 21, apparently inherited from a normal mother. The abnormal phenotype of the proband suggested the continuation of the investigation efforts. ArrayCGH and FISH were subsequently applied and showed a proximal duplication of about 11,3 Mb of 13q12.11-q12.3 and a proximal deletion of about 14,5 Mb of 21q11.2-q21.3. The child's der(21) was confirmed to be inherited from a maternal balanced translocation between 13q and 21q. This structural chromosomal abnormality exposes the mother to a high risk of offspring affected by unbalanced chromosomal disorder and could explain her infertility.

Conclusions: Without complete investigation of subtle rearrangements, genetic counseling is a real challenge. ArrayCGH analysis should be performed when the karyotype is apparently balanced but a chromosome anomaly is still suspected based on the clinical phenotype.

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E-P11.35

Patient with de novo 18q22.1-q22.3 deletion. A case report

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Laboratory evaluation of patients with developmental delay/ intellectual disability, congenital anomalies, dismorphic features and autistic disorders has changed significantly in the last years with the introduction of microarray technologies. Structural aberrations of chromosomes are associates with various syndromes, although there are a lot of chomosomal gains and losses that are not yet associated with a specific syndrome. Most of the cases are the novo, few cases are due to unbalanced rearraments and others are inherents. We present a three years-old boy derived from neuropediatric for genetics studies due to global developmental delay. He was born at 38 gestation week from no consanguineous healthy parents. Karyotype in leucocites was normal (46;XY). Comparative Genomic Hybridization (CGH-Array) with the Nimblegen CGX Cytogenetic Microarrays platform, suplied by PerkinElmer, was performed for the patient and parents. The CGH- Array was normal en both parents, but the patient presented a delecction of 2,40 Mb in 18q22.1-q22.3 chromosomical region (arr[hg19] 18q22.1-q22.3 (66,375,444-68,773,023)x1 Abnormal Male). The duplication is "de novo" and no previously described. These delection have 11 genes included (5 OMIM) and then with a high pathologic probability. . Chromosomic aberrations detected "de novo" and no previously described can be dificult to determine his pathologic potential, and it is necesary to have in mind the patient pathology and genes involved in the delecction/ duplication . In our case we believe that the chromosomal alteration detected, alone or in conjuntion with another chromosomal or enviromental cause will be responsible of the patient pathology.

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E-P11.36

Distribution of the Common TERT and TP53 Variants in Down Syndrome Children

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Introduction: The phenotype spectrum of Down syndrome (DS) related with the trisomy of chromosome 21 has a multi system associated features, but the exact mechanism of these abnormalities are not fully explained to date. To understand the role of common variants of TERT and TP53 genes on the diversity of DS phenotype, the frequency of these two variants was aimed to be investigated. The TERT gene rs2736100 A-C intronic variant is known with its association with various morbidities. The TP53 gene codon 72 Arg>Pro variant is related with altered TP53 protein functions. Allele distributions of these common variants were assessed between DS and control children.

Materials and Methods: DS (n:80) and control (n:32) subjects were included in this study. TERT gene rs2736100 A-C intronic variant and TP53 gene p.Arg72Pro (c.C215G) variant was determined with melting analysis of hybridization probes.

Results: The allele frequency of TERT and TP53 variants were not different between DS and control groups, however allele frequencies were higher than that of the highest population level. The TERT variant distribution was similar in the control group of children with homozygous Pro72 variant or Arg72 variant carriers. The variant distribution of TERT, however, was significantly lower in DS group of children with homozygous Pro72 variant compared to Arg72 variant carriers.

Conclusion: Similar distribution of the common variants of TERT and TP53 genes are implying that these variants may contribute to DS phenotype by more complex associations.

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E-P11.37

Enamel-renal syndrome: identification of two novel non-consanguinous families with mutations in *FAM20A* gene

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Amelogenesis imperfecta forms a group of inherited conditions having in common a defect in dental enamel formation on primary and/or secondary dentitions. It can be isolated or syndromic. When associated with nephrocalcinosis, the condition is designated as « enamel-renal syndrome » or « amelogenesis imperfecta type IG » (OMIM 204690), and is consecutive to biallelic mutations of *FAM20A* gene. The encoding protein is involved in the manufacture and maturation of enamel. However, the mechanism explaining the renal damage remains unclear. Rare, this autosomal recessive condition is often observed in consanguineous families. We report on 2 new patients from distinct and non-consanguineous families. The first, 14-year-old, had amelogenesis imperfecta and nephrocalcinosis without impact on renal function, accidentally

discovered at age 6 (abdominal pain). Two heterozygous mutations were discovered in *FAM20A*: c.1192G>T [p.(Asp398Tyr)] and c.1228_1229del [p.(Asp410Profs*5)]. The second, 6-year-old, had isolated amelogenesis imperfecta. His renal ultrasound examination was normal. Molecular analysis of *FAM20A* identified 3 heterozygous mutations: c.826C>T [p.(Arg276*)], c.1369A>T [p.(Lys457*)] and c.1517T>A [p.(Leu506Gln)]. These observations confirm that the emergence of rare diseases of autosomal recessive inheritance is also possible in non-consanguineous families. The absence of nephrocalcinosis in the second patient does not question the diagnosis of enamel-renal syndrome, and emphasizes that *FAM20A* mutations must also be investigated in isolated amelogenesis imperfecta. Nephrocalcinosis is inconstant, unapparent, and most often observed later in life. In enamel-renal syndrome, the risk for renal function justify the organization of a rigorous renal supervision.

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E-P11.38
Case report: A novel frameshift deletion in *ERCC6* gene causes Cockayne syndrome type B

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Cockayne syndrome (CS; OMIM 133540/216400) is a multi-systemic and progressive rare autosomal recessive condition. CS is member of the DNA repair disorders group, with a highly variable clinical phenotype, ranging from severe prenatal forms to mild cases with late presentations. Classical features include global developmental delay, post natal growth failure, feeding difficulty, microcephaly, cutaneous photosensitivity, dental, auditory (progressive hearing loss) and ophthalmological (pigmentary retinopathy, cataracts and enophthalmia) anomalies. CS is mainly caused by mutations in ERCC6 (OMIM 609413) and ERCC8 (OMIM 609412) genes.

Case report: We report a sporadic case of a girl with CS who presented with severe growth failure (3,5 kg at 5 years) - cachectic dwarfism, microcephaly, congenital cataract and aged appearance with prominent beaked nose and sunken eyes. Molecular genetic analysis of DNA from peripheral lymphocytes using NGS panel (4800 genes) revealed a novel frameshift deletion in homozygous condition - **NM_000124: c.1323delA (p.Gly442GlufsTer24) - in exon 5** of *ERCC6* gene. The Sanger sequencing test for the carrier status of both genitors is scheduled.

Conclusions: Our paper provides, to our knowledge, a detailed clinical presentation of a newly identified homozygous mutation in a girl affected with CS. This expands the mutational spectrum associated with this extremely rare genetic condition. This is the first case of CS identified and genetically confirmed in the South-West Romania.

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E-P11.39
Autosomal recessive oculodentodigital dysplasia: the third mutation identified in the *GJA1* gene

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Introduction: Oculodentodigital dysplasia (ODDD) is a rare condition characterized by typical facial appearance and variable findings of the eyes, teeth and fingers. Besides ocular manifestations like microphtalmia, microcornea, iris abnormalities and glaucome, small nose, microdontia, dental anomalies, syndactyly of the 4th-5th fingers (type III syndactyly), absent phalanx of the toes and dry hair can also be seen in individuals with this syndrome. ODDD is caused by mutations in the *GJA1* gene on chromosome 6q22-q24 and inherited in an autosomal dominant manner in the majority of the patients. However, in recent clinical reports, autosomal recessive ODDD cases caused by *GJA1* mutations were also described.

Case Report: Here we report a 14-year-old boy with microphtalmia, microcornea, narrow nasal bridge, hypoplastic alae nasi, prominent columnella, hypodontia and partial syndactyly of 2rd - 3th toes. These clinical findings were consistent with the diagnosis of ODDD, and a novel homozygous mutation, c.442C>T, *p.Arg148Ter* was determined in *GJA1* gene causing to premature stop codon. His healthy parents were found to be carriers for the same mutation.

Conclusion: *p.Arg148Ter* mutation in *GJA1* gene that encodes the transmembrane protein, connexin43 (Cx43) is responsible for the clinical features of the patient causing to premature stop codon and probably, leading to a truncated

non-functional protein. Up to now, autosomal recessive ODDD has been reported in eleven patients from six families but, only two of these cases were confirmed by molecular analysis. This is the third mutation described so far in the literature leading to autosomal recessive form of ODDD.

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E-P11.41

Holoprosencephaly, orofacial cleft and fronto-naso-orbital encephaloceles in a highly inbred patient: a new recessive disorder?

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Introduction: We describe a four-month-old Brazilian boy with semilobar holoprosencephaly, fronto-nasal encephaloceles and bilateral cleft lip and palate. Malformations included agenesis of the corpus callosum, abnormal cortical gyres, dilation of the aqueduct, bilateral endolymphatic sac, bilateral cystic cocci-vestibular malformation and a cribriform defect. The 3D TC craniofacial images showed abnormal frontonasal transition region, with a bone bifurcation and partial agenesis of nasal bone. The trunk and upper and lower limbs were normal.

Methods: G-banding karyotype and SNP-array.

Results: Karyotype was normal. SNP-array showed no copy-number alterations but revealed a high rate of regions of homozygosity with normal copy number (ROH). The coefficient of inbreeding is 25%, suggesting that the proband was a result of a parent-offspring or a full-siblings relationship, which significantly increases the risk for an autosomal recessive disorder. Searches in the Genomic Oligoarray and SNP array tool for recessive disorders did not reveal any gene or disorder within the patient's ROH that matched his clinical features. *STIL* and *FGF8*, two recessive holoprosencephaly genes, do not map within our patient's ROH.

Conclusion: To our knowledge, this rare association of holoprosencephaly with fronto-naso-orbital encephaloceles without limb anomalies has never been reported before. No known recessive disorder matches our patient's clinical symptoms. These results suggest that a recessive mutation in a new gene could be responsible for this rare association. It is also possible that these atypical clinical findings are the result of a multiple recessive or complex molecular diagnosis.

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E-P11.42

Holopsosencephaly could be the extreme phenotype ofRTTN mutations

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RTTN was involved in a recessive autosomal polymigrogyria (PM) when it was first described in 2012. More recently, in 2015, RTTN was also involved in microcephalic primary dwarfism (MCPH) and in microcephaly with simplified gyral pattern. Function of the protein Rotatin is still unknown but its interaction with another protein of the centriole, the essential organelle required for cell division, has already been described. We report the clinical, neuroimaging and neuropathological data from 2 foetuses from a consanguineous couple: the 1st foetus showed a severe microcephaly and the pregnancy was terminated at 21WG. Sonogaphic follow-up of the second foetus reveled a holoprosencephalic aspect and the pregnancy was terminated at 16WG Neuropathological examination found a lissencephaly and ventricular heterotopia in the 1st foetus and an "incomplete holoprosencephaly" and neuronal migration anomaly in the 2nd foetus. Previously described homozygote missense mutation in exon 23 of RTTN was found. We compared the phenotype of those 2 foetuses with 3 new patients of our serie and the patients previously described in the literature.

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E-P11.43

Unusual clinical and laboratory findings in a case with Ochoa syndrome

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Introduction: The Ochoa or Urofacial syndrome (UFS) is a rare autosomal recessive disease caused by mutations in either *HPSE2* or *LRIG2* genes. UFS is characterized by the association of abnormal facial expression with a lower urinary tract and bowel dysfunction. The patients with this syndrome made a typical painful grimace when they tried to smile. Progression to urinary incontinence, megacystis, vesicoureteric reflux, hydroureteronephrosis, and renal failure if not early recognition of the disease is unavoidable. We report the first Bulgarian case of this rare syndrome.

Materials and Methods: The patient, a seven-year-old girl, was referred to genetic evaluation because of the severe mental retardation and autistic behaviour. She was born at term after complicated pregnancy. The parents are third cousins. From early childhood the girl suffered from frequent vomiting episodes during respiratory infections and constipation. Physical examination revealed microcephaly and mild mandibular prognathism. Serum lactate levels were significantly elevated. Metabolic and mitochondrial DNA testing results were normal. In an attempt to establish the diagnosis Whole exome sequencing was carried out.

Results: A homozygous mutation was identified in exon 3 of the *HPSE2* gene: c.C457T:p.R153X. Recurrent urinary tract infections were proven as well as a peculiar inverted facial expression was noticed after receiving results from DNA analysis.

Conclusions: Unusual clinical and laboratory findings in our case led to delay in diagnosis. The characteristic facial expression allows early detection of this rare disorder. Early recognition of UFS and adequate prophylaxis and treatment can prevent upper urinary tract damage and renal failure.

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Possible effect of *IGFR1* gene on macrocephaly in a patient with unbalanced 6;15 translocation with 6p25 deletion and 15q26 duplication

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We report a 2.5 years old girl with a 1877kb loss of terminal 6p25.3 and 8849 kb gain of distal 15q26.1 to ter due to an unbalanced translocation inherited from the mother carrying the balanced translocation. She is the second liveborn from a 30 year old healthy mother. Prenatal ultrasound showed cystic cysterna magna and MRI follow up revealed ventricular dilatation. She was born at 38+3 weeks gestation. Macrocephaly and buphthalmos was noted at birth. She was operated for glaucoma. She was evaluated in pediatric genetics clinic for dysmorphic facial features and developmental delay. The heterozygous loss in 6p25.3 resulted in loss of 6 OMIM annotated genes including the transcription factor *FOXC1* gene. This gene plays an important role in eye, brain and heart development. Microdeletions of this region are known to be associated with microcephaly, distinct clinical features, like sensorineural hearing loss, anterior chamber eye defects, cardiac defects and developmental delay. The gain in 15q26.1-3 includes 17 OMIM genes including *IGF1R* gene, which we speculate explains the macrocephaly, instead of microcephaly. This patient demonstrates the combination of two copy number variants where the loss (deletion) predominates the phenotype and additional findings are likely to be addressed to the genes in the gain (duplication) segment.

We conclude that patients with a relatively well-delineated microdeletion syndrome as 6p deletion should be thoroughly evaluated for additional copy number variants when presenting with atypical or unusual phenotypical findings with emphasis on all genes involved in the imbalance.

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E-P11.45
Identification of novel mutation in KMT2D gene in a patient with Kabuki syndrome and borderline chromosomal instability

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Kabuki syndrome (KS) is a rare, multiple congenital anomaly syndrome. It is caused by mutations in KMT2D and KDM6A genes that are involved in histone methylation and thus leading to epigenetic dysregulation. KS is characterized by distinctive facial features, intellectual disability, postnatal growth retardation and various structural anomalies. Other findings such as increased susceptibility to infections and autoimmune disorders have also been observed. Cancer predisposition in KS remains uncertain and it has been described in less than 10 cases so far.

We report 10-year-old boy with typical facial features of KS, fetal fingertip pads, intellectual disability, complex heart defect, minor skeletal anomalies, hearing loss and short stature due to growth hormone deficiency. He also suffered from IgA and IgG hypogammaglobulinemia and was operated due to neuroblastoma in the 3rd month of his life.

No imbalance was detected in array CGH. Targeted gene sequencing revealed a novel, truncating variant (c.2063_2064del/ p.Arg688Profs*6) in exon 10 of KMT2D gene which confirmed diagnosis of KS in the patient. Additionally performed assay showed bleomycin- induced chromosome breakage at a rate of bordeline value (0,74 vs 0,8, which was a cut-off value adopted in our facility).

Histone methyltransferase encoded by KMT2D gene is a major epigenetic factor which interacts with other protein complexes regulating expression of multiple genes and many cell functions. The present observation of chromosome breakage in a patient with Kabuki syndrome may therefore indicate its significance for chromatin stability and affect clinical characteristics and management of the disease.

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E-P11.46
Two Novel *KMT2D Gene* Variants in Kabuki Syndrome

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Kabuki syndrome is a rare, multiple malformation disorder characterized by distinctive facial appearance, cardiac anomalies, skeletal abnormalities, joint laxity, short stature, prominent finger pads and intellctual disability. Recent studies reported patients with Kabuki syndrome caused by variations in *KMT2D* and *KDM6A*, two interacting chromatin modifier responsible for 56-75% and 5-8% of the cases, respectively. Two patients were referred because of congenital anomalies and dysmorphic features. They were borned to healthy non-consanguineous parents. We clinically diagnosed two cases of Kabuki syndrome due to typical features. Sequencing analysis of *KMT2D* gene was performed and two novel variations were identified. Two heterozygous variants were found after parental carrier testing, namely c.438G>A [p.(S146S)] and c.5674C>T [p.(Gln1892X)]. *In silico* tools (Mutation Taster, SIFT and PolyPhen) predicts these mutations as potentially deleterious. Parental carrier testings confirmed that these variants occured as *de novo* events. Clinical manifestation of these two cases assessed as moderate or mild form of the disease. Therefore, fuctional studies and additional case reports are required to understand the affect of these variations and genotype-phenotype correlation.

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Kabuki syndrome - long-term follow-up of five cases

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Kabuki syndrome (KS) is characterized by distinctive facial features, minor skeletal anomalies, fetal pads, intellectual disability (ID) and postnatal growth deficiency. Cardiac, renal and skeletal defects are sometimes associated. KMT2D and KDM6A are the only genes known to determine KS. We present 5 cases with KS in order to illustrate particularities identified, but also to discuss the changing of the clinical picture in time. All our cases are males, represent sporadic cases and have been confirmed by mutation analysis of KMT2D. Growth charts and evolution in time will be provided. Case 1: marked postnatal growth retardation, obesity, typical face, soft skin, fetal pads, severe vesico-ureteral reflux leading to chronic renal failure, moderate/severe ID with ADHD; the mother has typical face and fetal pads (no mutation); Case 2: normal growth, obesity, typical face, soft skin, fetal pads, heart defect, unilateral renal agenesis, nephrocalcinosis, moderate ID; Case 3: normal growth, situs inversus, typical face, fetal pads, cardiac and renal defect, severe ID with behavioral disturbance; Case 4: normal growth, obesity, typical face, soft skin, fetal pads, gynecomastia, cardiac and renal defect, moderate/severe ID with ADHD; the mother has typical face and fetal pads (no mutation); Case 5: postnatal growth

deficit, presternal obesity, typical face, dry skin, fetal pads, cardiac and renal defect, mild ID with marked ADHD. In conclusion, we have identified particular features in our KS patients (obesity, soft skin, ADHD, situs inversus). All of them have cardiac and renal defects. ID was present in all cases (severe in 4/5 cases).

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Kagami-Ogata syndrome: phenotype of a non-Japanese patient with microdeletion of imprinted region

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Kagami-Ogata syndrome (KOS) originates from an absence of a maternal copy of imprinted region 14q32.3, where a nonexpression of a *MEG3*-gene plays a critical role. About 2/3 of the KOS-patients show paternal uniparental disomy 14, the rest either an epimutation or a deletion of the critical region. Majority of the published KOS patients are of Japanese origin and non-Japanese patients have been reported rather seldom, as well as the phenotypes with microdeletion. An Array-CGH analysis in our patient showed a microdeletion of 670 kb in the critical region 14q32.2, verified also by FISH. An MLPA analysis confirmed a heterozygous deletion of *MEG3*-, as well as *RTL1*- and *DLK1*-genes, and showed a hypermethylation pattern of *MEG3*-gene - the evidence that the deletion originated at the maternal allele. The healthy mother showed no microdeletion and normal MLPA (de novo microdeletion in daughter). Our patient showed except of the 14q32.3 deletion also a deletion of 1,2 Mb in region 16p13.11, however the possible phenotypes of the absent genes do not correlate with the phenotype of the patient. The mother does not carry this second microdeletion and the father was unavailable for the analysis. The patient had been evaluated in her age of 1,5y and 5y. She showed a global developmental delay with severe hypotonia, typical costovertebral anomalies (significant congenital scoliosis, pectus excavatum, “coat-hanger" ribs), facial dysmorphy, adducted thumbs and an abdominal muscle defect. She needed no ventilation in infancy, but had an absent sucking reflex, massive sialorrhoea and a PEG till 5y.

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Chromosomal Variants in Klinefelter Syndrome

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Introduction: Klinefelter syndrome (KS) describes the phenotype of the most common sex chromosome abnormality in humans (1/600 newborn males). The most widespread karyotype in affected patients is 47,XXY, but various other karyotypes have been described. The aim of this study was to examine the karyotypes of a group of our patients suspected of having Klinefelter's syndrome.

Materials and Methods: Since 1993, 98 adult KS patients have been evaluated in the Laboratory of Cytogenetics and Molecular Genetics Clinic of Hematology, Clinical Center of Serbia. Cytogenetic analysis was carried out on metaphases obtained from phytohemagglutinin-stimulated peripheral lymphocytes using a standard procedure. Fluorescence *in situ* hybridization (FISH) analysis was performed on peripheral blood specimens. Vysis CEP X/Y- alpha satellite DNA probes were used to detect X and Y chromosomes.

Results: We identified KS with the ‘classical’ karyotype in 77 patients, KS with a mosaic karyotype- 47,XXY/46,XX in three and 47,XXY/46,XY in four (confirmed by FISH), KS with the ‘classical’ karyotype together with other autosomal chromosomal abnormalities in six, and KS with other numerical sex chromosome abnormalities-48,XXYY in two (confirmed by FISH) and 47,XYY in six patients.

Conclusion: In our group of KS patients most had the ‘classical’ karyotype, but some men had diverse other karyotypes. It is important to determine the nature of the karyotype of every male with clinical features of KS in very early childhood in order to initiate an adequate, personalized, medical approach.

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E-P11.50

A five-year-old boy with Laurin- Sandrow syndrome with micro-duplication on 7q36.3, duplicating a part of the LMBR1 gene

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Laurin-Sandrow syndrome (LSS) (OMIM # 135750) is a rare polydactyly syndrome characterized by mirror image polysyndactyly of hands and feet, nasal defects, and often accompanied by fibula and ulna duplication along with absence of tibia and radius. It has an autosomal dominant pattern of inheritance and is caused by heterozygous mutations in an SHH (OMIM 600725) regulatory element (ZRS) that resides in intron 5 of the *LMBR1* gene (OMIM 605522). Microduplications were detected in 3 families with Laurin- Sandrow syndrome in the 7q36 chromosomal with lengths varying from 16 to 75 kb. We report on a five-year-old Iranian boy of distantly related parents, presented with prominent forehead and upturned flat tip nose, syndactyly of all fingers and toes, hypoplastic nails, preaxial polydactyly of both feet and hypoplastic nails of feet. We identified a de novo 86 kb micro-duplication on 7q36.3, encompassing exons 5 to 15 of the *LMBR1* gene including the ZRS element by array comparative genomic hybridization (aCGH). The duplication was verified by real-time quantitative PCR (qPCR).

Key words: Laurin- Sandrow syndrome, polysyndactyly, nasal defect, LMBR1

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E-P11.52

A 10q21.2 microdeletion identified in a patient with moderate intellectual disability and dysmorphic facial features

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Introduction: Interstitial deletions in 10q21.2-q22.2 are rare and the phenotype is not well characterized. Important genes in this region include: RTKN2 involved in lymphopoiesis, ARID5B affecting cell growth differentiation of B-lymphocyte progenitors, adipogenesis and liver development and ZNF365 involved in morphogenesis of basket cells in the somatosensory cortex during embryogenesis and in the oligodendrocyte differentiation during postnatal growth.

Materials and Methods: Investigation was performed with whole-genome oligonucleotide microarray CGH analysis using clinical 60K microarrays from Oxford Gene Technology (CytoSure ISCA v2).

Results: The patient, a 6-year-old girl is the only child of nonconsanguineous, caucasian parents. The pregnancy was physiological and a routine ultrasound during pregnancy did not show any abnormalities. Delivery was by cesarean section at 39th week of gestation. The child's birth weight was 3750 g, length 54 cm and the occipitofrontal head circumference 35 cm. The patient achieved sitting without support at the age of 6 months, at the age of 18 months she started walking. At the age of 4 years the patient was referred to the genetic clinic for evaluation because of moderate intellectual disability and dysmorphic facial features. Her mother had similar dysmorphic facial features and intellectual disability. Microarray CGH analysis showed a deletion at 10q21.2 in the patient and her mother. The identified deletion included RTKN2, ARID5B (exons 8-10) and ZNF365 (exons 1-5).

Conclusion: For the first time, we describe the clinical characteristics of two patients with a deletion of RTKN2, ARID5B and ZNF365.

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E-P11.55

Challenges in the modern genetic diagnostics of a child with a malformative syndrome and intellectual disability

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Introduction: Advances in genomics and development of new genetic tests allow the diagnosis of rare malformative syndromes that may not be diagnosable with other methods. In some cases, however, they generate confusing results that are difficult to interpret or use in a way that improves clinical care.

Materials and Methods: We report on a 7-year old girl with moderate intellectual disability, psychomotor and speech delay, hypomimic face with dysmorphic features, hypodontia, axillary and inguinal linear

hyperpigmentations, horseshoe kidney, clinodactyly and abnormal toes. At birth severe muscle atonia and areflexia were observed. Cytogenetic analysis revealed normal karyotype. At age 6 whole-genome analysis using the HumanCytoSNP-12Beadchip and whole-exome sequencing using Illumina MiSeq were performed.

Results: Molecular karyotyping revealed 46,XX, arr13q14.11 microduplication (440 Kb) with paternal origin, considered to be benign variant, based on the normal phenotype of the father and reports in healthy controls. NGS detected heterozygous variants in several genes, but only 4 (*WNT10A, ANO5*, *KCNE2*, *POLG*) were classified as "pathogenic" or "likely pathogenic" with possible autosomal dominant expression. However, none of these gene variants could fully explain the phenotype of the child and the next step in the interpretation of the results will be sequencing of the parents for seeking these mutations.

Conclusions: Reaching an accurate genetic diagnosis in children with multiple congenital abnormalities / intellectual disability syndromes is still a great challenge. In many cases, a detailed family history and genetic evaluation of additional family members is essential in determining the significance of genetic variants identified in the patient.

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E-P11.56

A mosaic double aneuploidy: mos 45,X/47,XX,+18/46,XX with mild phenotype

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Introduction: Mosaic trisomy 18 and monosomy X concordance is a very rare genetic phenomenon. There is a small number of cases in the literature. Here, we present an 18-year-old girl with mild phenotype.

Clinical Report: An 18-year-old female patient followed-up to the psychiatric department due to anxiety disorder and nocturnal enuresis. She had no mental retardation but her school performance was low. She had short stature, hypotelorism, trident posterior hairline, cubitus valgus, atrial septal defect and irregular menses with normal uterine and ovarian morphology. Her gonadotropin and sex hormone levels was within the normal range.

Materials/Methods: Karyotype analysis was performed from two separate lymphocyte cultures from the peripheral blood sample and fibroblast culture from the antecubital skin. Additionally, we evaluated the chromosome X and chromosome 18 numbers with fluorescent in situ hybridization (FISH) technique.

Results: A total of 100 metaphases were counted from the lymphocyte cultures of the patient. There were 68 metaphases with 47,XX,+18; 27 metaphases with 45,X; and 5 metaphases with 46,XX karyotype. FISH analysis was confirmed the result. The fibroblast culture revealed 21 metaphases with trisomy 18 and 51 metaphases with monosomy X without normal metaphases.

Conclusions: Although the patient showed a high level of trisomy 18 cell group in the lymphocyte culture, she had more similar Turner phenotype rather than trisomy 18 phenotype. There is no correlation between the trisomy 18 ratio in lymphocyte or fibroblast culture and the phenotype, as can be seen in this case and in other defined cases in the literature.

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A variant outside the catalytic domain of SMARCA2 is associated with a phenotype distinct from Nicolaides-Baraitser syndrome

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Introduction: Mutations in *SMARCA2* gene encoding a chromatin remodeling gene are responsible for Nicolaides-Baraitser syndrome (NCBRS) presenting with intellectual disability (ID), seizures, short stature, and a recognizable pattern of facial dysmorphic features. The majority of NCBRS causative *SMARCA2* mutations affect the ATPase domain (exon 15- 25). To date, *SMARCA2* variants outside the ATPase domain have been identified by whole exome sequencing (WES) in two individuals lacking the typical NCBRS facial features.

Patient and Methods: Here, we report a 6-year-old individual harboring a *de novo* variant detected by WES in the *SMARCA2* gene not affecting the catalytic domain (p. Leu529Val; exon 9). This individual presented with frontal bossing, short palpebral fissures, downturned nasal tip with hypoplastic alae nasi, thin upper lip, moderate ID and Arnold-Chiari type 1 malformation.

Results and conclusions: The facial features were not consistent with NCBRS dysmorphic features. Although further cases need to be investigated, the individual herein

described suggest that *SMARCA2* mutations falling outside the catalytic domain might result in a phenotype that is distinct from NCBRS.

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E-P11.58

17q22 microdeletion detected by array-CGH leading to NOG-related symphalangism spectrum disorder (NOG-SSD)

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17q22 microdeletions involving the NOG gene have been reported to cause the NOG-related symphalangism spectrum disorder (NOG-SSD). NOG-SSD is mainly characterized by symphalangism and the ankylosis of the joints in fingers or toes. Other findings reported in the literature in relation to 17q22 microdeletion are microcephaly, urogenital anomalies (absence of uterus / vagina, cryptorchidism, penile chordee, duplex renal system, small penis), mild/moderate intellectual impairment, speech stress, cervical and/or other vertebral fusion/defects and hypogonadotropic hypogonadism.

A thirteen years old male referred us because of speech delay and short fifth finger. Her parents were nonconsanguineous and healthy. He had dysmorphic features including bilateral ptosis, epicanthus, deep-set small eye, pollybeak deformity, low-set ears, high narrow palate, shortened philtrum, micrognathia, arachnodactyly, long neck, bilateral shortening of the 5th middle phalanx of the hand and bilateral syndactyly of toes 2-3, pectus carinatum, and dorsal hypopigmentation, hyperopia. The patient was operated for undescended testis at the age of five. There is only one seizure history occured at the age of 13. G-banding karyotype using peripheral blood was normal. Array CGH analysis was performed on Agilent 4x180K CNV+SNP oligo-array by manufacturer's protocol. 3977 kb sized copy number deletion arr[hg19] 17q22 (51,848,499-55,825,448) x1 was identified in our patient.

To our knowledge, 17q22 microdeletions involving the NOG gene has been reported extremely rare previously. The Array-CGH result of our patient 17q22 microdeletion contributed to the genotype-phenotype correlations associated with this chromosomal aberration.

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De novo 15q26.2-q26.3 deletion in a patient with oculo-auriculo-vertebral spectrum

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Normal 0 14 false false false IT JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"-Tabella normale"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin:0in; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria; mso-ascii-font-family: Cambria; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Cambria; mso-hansi-theme-font:minor-latin;} Oculo auriculo vertebral spectrum (OAVS, MIM164210) is a rare disease due to an altered development of the derivatives of the first and second branchial arch. We observed a five-year-old patient with growth retardation, hemimandibular and maxillary hypoplasia, microretrognathia, preauricular pits, corneal lipodermoid, cataract, vertebral anomalies and congenital heart defects. Simple and complicated dental caries and mild developmental delay were also present. Thyroid profile and hormonal analysis of *IGF1* gene were in normal ranges, like analysis of bone age data which corresponded to chronological age. Research of microdeletions and microduplications through SNP-array analysis (Cytoscan HD array) identified two different genomic rearrangements: a de novo deletion of about 5 Mb on chromosome 15q26.2-q26.3 and a maternally transmitted deletion of 120 Kb on chromosome 9p22.1. Terminal deletion of long arm of chromosome 15 are a rare cause of shorth stature (prenatal and postnatal growth retardation), and are associated to microcephaly, skeletal anomalies, facial and auricular dismorphic features. The same chromosomal region has been previously involved in OAVS by linkage analysis in a three-generation family. This study reports the association between deletion of chromosome 15q26.2-q26.3 and OAVS, adds further genetic heterogeneity to this condition, suggests the presence of one or more candidate gene/s in this region, confirming the importance of array-based studies in patients with OAVS. <!--EndFragment-->

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E-P11.60
14q22 Copy Number Variations encompassing *OTX2*, new cases widening the phenotype

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Introduction: *OTX2* (14q22.3) is key in craniofacial and sensory organ development. 14q22.3 Copy number variants (CNV) affecting *OTX2* have been identified in patients with a great variety of phenotypes, from autosomal dominant retinal dystrophy to the otocephaly-dysgnathia-complex (ODC) and hemifacial microsomia (HM), being the most frequent phenotype severe eye defects (microphthalmia/anophthalmia); with or without cerebral malformations and/or pituitary abnormalities with inter and intra-familial variability and incomplete penetrance.

We present the phenotypic findings of a 3 generation family displaying all the *OTX2* associated phenotypes, and a case presenting neuroblastoma.

Patients and Methods: Comparative genomic hybridization (aCGH), Agilent1 60K Human array (ISCA design). Revealed in the Agilent G2565BA microarray scanner (Plateforma G_enomique, Nantes) and analyzed by Agilent Cytogenomics 3.0 software. Genomic position: UCSC Genome Browser on Human Feb. 2009 (GRCh37 / hg19) Assembly43,

Results: Family A: 2.15Mb deletion (14q22.2q22.3) *OTX2* being the only pathology associated OMIM gene.

Family B: 370Kb deletion (14q22.3) affecting exclusively *OTX2*.

Table 1 and figures 1 and 2 show the cases´ phenotypes.

Conclusions: First family case of a 14q22.2q22.3 deletion exhibiting all *OTX2* known phenotypes

Neurobasltoma could be an additional *OTX2* phenotypic characteristic, and supports the possible involvement of *OTX2* in nasal tumours pathogenesis.

Phenotypic variability is key in the counselling of these families.

Table 1.Clinical findings on the new described patients presenting a deletion on 14q22 region

Family / patient	A/**	A/I.1	A/II.2	A/II.3	A/II.4	A/II.6	B/II.1
14q		22.2q22.3	Not analyzed	22.2q22.3	22.2q22.3	22.2q22.3	22.3

Table (continued)

Family / patient	A/**	A/I.1	A/II.2	A/II.3	A/II.4	A/II.6	B/II.1
	Not analyzed						
CNV type, size and break points	NA	Del, 2.15Mb, 55386907-57537913	NA	Del, 2.15Mb, 55386907-57537913	Del, 2.15Mb, 55386907-57537913	Del, 2.15Mb, 55386907-57537913	57166523-57537913
Gender	M	M	M	M	M	F	F
Prenatal findings	NO	NO	Agnatia	NO	NO	Ventriculomegaly	Small for gestational age
Age at lastassessment	NR	32yr	21w*	28w*	10yr	11m	12yr
Strucutral eye anomalies	NO	NO	NO	NO	NO	Microphthalmia, iris coloboma BE	NO
Optic nerve defects	NR	NR	NR	NR	NR	Nistagmus under investigation	NR
Retinal dystrophy	NR	Maculopathy	NR	NR	NR	Nistagmus under investig	NR
Pituitary strucutral anomalies	NR	NR	NO	NO	pitutary mass	NO	Hypoplasia
GH deficit	YES	NR (normal stature)	NA	NA	Normal growth	short stature under investigation	YES
Other pituitary hormone deficiencies	NR	NR	NA	NA	NR	NR	Hypotiroidism
Brain malformation	NR	NR	NO	NO	NR	Ventriculomegaly, cerebellar hypoplasia, small corpus callosum	NO
Neurodevelop-ment	Learning difficuleties	Normal	NA	NA	Autism and learning difficulties	Normal	Normal
ODC	NR	Micrognatia	Agnatia, microstomia, caudally positioned ears, absence of external auditory canal	Micrognatia, microstomia, small tongue	Micrognatia	NR	Micrognatia
Facial asymmetry	NR	NO	NO	Jaw asymmetry	NR	NO	NO
Velopharyngeal anomalies	NR	NR	NO	NO	NR	Laryngomalacia	NR
Microcephaly	NR	NO	NO	NO	NR	NO	NO
Digit anomalies	NR	NO	NO	NO	fixed position of 5th finger	NO	NO
Kidney anomalies	NR	NR	NO	NO	NR	NR	NR
Heart anomalies	NR	NR	NO	NO	NR	NR	NR
Genital anomalies	NR	NO	NO	NO	NR	NO	NO
Hearing loss	NR	NO	NA	NA	NR	NO	NO
Dysmorphism	NR	NO	YES	YES	NR	NO	high forehead
Other	NR	NO	NO	NO	NR	NO	NEUROBLAS-TOMA

F. Blanco-Kelly: None. **H. Stewart:** None. **D. Shears:** None.

E-P11.62
Pre- and postnatal diagnosis of microchromosomal abnormalities in the population of Sverdlovsk Region (Russian Federation)

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Introduction: As many of hereditary diseases and syndromes diseases are characterized by territorial and ethnic heterogeneity it is very important to choice optimal methods for studying of pathology in particular population.

Materials and Methods: We present a genetic examination of 3359 samples (2012-2017 y.). 2,947 prenatal samples of fetuses (villus chorionic, amniotic cells, cord blood) were analyzed by BACs-on-Beads technology (PerkinElmer, Finland). 412 samples of whole blood from children with clinical signs of chromosomal abnormalities, who were born in the same period were performed by multiplex ligase amplification of probes (MLPA) (MRC-Holland, Netherland). DNA was examined for aneuploidy at 13, 18, 21, X, Y chromosomes and 9 microdeletion syndromes.

Results: The number of microstructural chromosome disturbances was detected more often for children than in the fetal materials (17.5% vs. 1.5%).

Syndromes	Region	Detected prenatal, (n=2947)	Detected postnatal, (n=412)
Di George	22q11.2/ 10p14	5	12
Cri-du-chat	5p15.3-15.2	3	4
Williams -Buren	7q11.2	1	10
Prader Willi/Angelman	15q11-q12	-	8
Wolf-Hirschhorn	4p16.3	-	4
Smith -Magenis	17p11.2	-	2
Miller-Dieker	17p13.3	-	1
Pallister-Killian	12p	1	
Microdeletion/microduplication not associated with known syndromes		35	31
Total (%)		45 (1,5)	72 (17,5)

Conclusions: The existing criteria for selection of pregnant women for prenatal diagnosis (the combination of anamnestic, biochemical and ultrasound data) are ineffective for diagnosing of micro anomalies of chromosomes. The MLPA or BoBs technologies are a reliable method of searching for microchromosomal rearrangements for the diagnosis of genetic syndromes for patients need medical genetic counseling.

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E-P11.63
Clinical assesment importance in an asymmetrical overgrowth case in the context of New Generation Testing

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Introduction: Whole exome sequencing (WES) uses Next Generation sequencing technology (NGS) to identify mutations in all the coding regions of an individuals' genome. WES is useful when an individual is presenting multiple phenotypical characteristics that are not suggestive for a specific syndrome. We present a case of a six-year-old boy presenting with upper limb asymmetry, hemangioma of the distal phalanx of the index finger on the left hand, hemihypertrophy and extended hemangioma of the left hemithorax. The abnormal vascularization pattern of the left hemithorax was observed in the third trimester of pregnancy. Based on the clinical presentation, our suspicion was of Proteus syndrome.

Material and Method: Due to genetic heterogeneity of Proteus syndrome, WES was performed for genetic confirmation.

Results: The WES analysis was unable to confirm Proteus syndrome or other cause for the child's phenotype, due to possible somatic mosaicism or technical limitation of the method. Noteworthy two incidental findings responsible of long QT syndrome and hypertrophic cardiomyopathy were reported.

Conclusion: A very important issue in communicating WES results are incidental findings. They represent mutations discovered by WES that can be associated with diseases which can affect the individual throughout his life. In our case, although the test didn't identify a genetic cause for the patient's phenotype, the incidental findings suggest that cardiological monitoring could be life-saving. Also familial genetic testing of above mentioned mutations is adviced.

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E-P11.64
Unexpected phenotype in a frameshift mutation of *PTCH1*

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Gorlin syndrome, also known as Basal cell nevus syndrome (BCNS #109400), is a rare autosomal dominant genetic disorder. The penetrance of BCNS is complete and the expressivity is variable. The most characteristic clinical manifestation is the development of multiple basal cell carcinomas at a young age; other common findings are: odontogenic keratocysts, calcified falx cerebri, skeletal anomalies, palmoplantar pits, macrocephaly and dysmorphisms. BCNS is mainly caused by mutations in *PTCH1* gene, the "protein patched homolog" gene: an onco-suppressor gene that maps at 9q22.32 region. Other genes that can be involved in the disease are *SUFU* and less commonly *PTCH2*. Those genes participate in the "sonic hedgehog" (SHH) signaling pathway. A disease related to BCNS is the 9q22.3 microdeletion syndrome. This syndrome has an overlapping clinical phenotype with the BCNS, but it can also present other possible findings in addition: metopic craniosynostosis, pre and post-natal overgrowth, obstructive hydrocephalus, developmental delay, intellectual disability and seizures. This condition is caused by the deletion of a genome region containing the *PTCH1* and the *FANCC* genes. Here we report the case of a 11 years old girl that came to our attention for overgrowth, dysmorphic features of the face and craniosynostosis, but with a normal intellectual and motor development. We present this case because it is the first one with a *PTCH1* mutation (p.Val502Glyfs*13) and a 9q22.3 microdeletion syndrome phenotype. This finding may strengthen the importance of the role of the *PTCH1* gene especially regarding the metopic craniosynostosis.

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E-P11.65
Phenotype characterization in patients with Noonan syndrome

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RASopathies are group of autosomal dominant disorders that are phenotypically similar. They are caused mostly by mutations in the RAS/MAP kinase signaling pathway. According to the clinical presentation and specific mutation, they are distributed in several subgroups. Phenotypic changes include facial dysmorphism, short stature, cardiac defects, haematological disturbances and variable neuropsychiatric problems. The mutations in PTPN11 gene are most commonly presented, followed by mutations in other genes - KRAS, HRAS. SOS1, etc. We present ten patients with clinical phenotype of Noonan syndrome. Phenotypic presentation is consistant with the diagnosis and include short stature, variable cardiac defects, skin and hair changes, genital anomalies in some. Facial changes include hypertelorism, antimongoloid eye slanting, long philtrum, dental maloclusion, low set ears, pterigia, etc.Two of the patients had hearing impairment. Molecular analysis was performed by the NGS method including genes: NRAS, ROT1, SCHOC2, HRAS, CBL, KRAS, PTPN11, SPRED1, MAP2K1, NF1 and MAP2K2. In 60% of evaluated patients mutations in PTPN11, RAF1 and KRAS genes were found. In four patients with typical NS features no mutations were found so far. Intelectual disability varies in all patients, ranging from minor to profound. Our findings indicate that mutation distribution in RASopathies are similar to those in other studies. Further molecular evaluation is needed in order to reveal all mutations in patients with NS. Although similar in clinical presentation, patients with RASopathy syndromes reveal some distinctive phenotype features that can direct the diagnostic procedure.

E. Sukarova-Angelovska: None. **M. Kocova:** None. **R. Kacarska:** None. **B. Lee:** None.

E-P11.66
Complex constitutive rearrangement involving chromosome 15 in a girl with postnatal growth and developmental delay

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Introduction: Rearrangements affecting chromosome 15 are rare and affected patients show a variety of nonspecific features, including complex congenital malformations, growth deficiency, and developmental delay.

Material and Methods: Here, we reported a 2 years old girl with postnatal growth delay, short stature, brachydactyly, triangular face, and facial dysmorphisms with prominent forehead, hypertelorism, bulbous nasal tip, long philtrum, thin upper lip, and micrognathism. Physical examination revealed height of 71 cm (<4rd) and weight of 7000 g (<3rd). G-banding analysis at a band resolution of >550 was performed, followed by FISH using probes 15q11-13 (SNRPN) for Prader-Willi/Angelman and 15q26.3 for internal control.

Results: The karyotype showed a constitutive chromosomal aberration, 46,XX,r(15)[64]/46,XX,r(15)dup(15)[16]/47,XX,+r(15)[5]. FISH analysis confirmed the karyotype results and showed two more different cell lines 46,XX[9]/45,XX,-15[6]. In r(15) was detected the absence of the 15q26.3 signal resulting in a genetic material loss, region that harboring *IGF1R* gene responsible for biological activity of IGF1.

Conclusions: Ring chromosome results from breakage in both arms of a chromosome, with fusion of the points of fracture and loss of the distal fragments. In this context, a ring induces chromosomal instability, which in turn generates a diversity of cell lines harboring different chromosome configurations. In the case described here, we hypothesized that a 46,XX zygote acquired a r(15) leading to the instability subjacent to the other cell lines that had ring duplication, monosomy 15, trisomy 15, which included r(15), most likely due to different approaches to restore balanced genome in the cells, such as trisomy rescue and UPD.

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E-P11.67

Ring X chromosome In a male with short stature and hypospadias

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Ring X is a rare chromosomal anomaly mainly seen in females with turner syndrome. In males it is extremely rare because nullisomy for X chromosome material is not compatible with survival and induce an early abortion. Only two cases of male with ring chromosome X and presenting a short stature as the only phenotypic abnormality (Ogata et al. 1990 and Ellison et al. 2002) were previously reported. We report here a new case of a two years old male with ring chromosome X characterized using Karyotype, FISH and 180K array Comparative Genomic Hybridization and presenting a short stature and hypospadias, without dysmorphic features or any psychomotor developmental delay. Molecular investigations showed that ring X chromosome in this patient presents a terminal deletion of 923 Kb including SHOX gene and totally located on the pseudoautosomal region 1 (PAR1) and an inverted duplication of 2.3 Mb proximal to STS gene. The non-loss of X chromosome material outside the PAR region explain the compatibility with survival in our case because the absence of functional nullisomy. Loss of SHOX gene is presumably common with the two previously reported cases and is the cause of short stature. However, hypospadias was not reported in the previous reported cases and may be due to the associated duplication outside PAR1 region and including particularly PRKX gene coding for a protein involved in urogenital system morphogenesis.

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E-P11.68

Variant in RNF113A gene, links neurodevelopmental pathway and dna repair pathways to severe congenital malformations

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RNF113A is an intronless gene that encodes a protein with a Ring type Zinc finger domain that seems to be involved in DNA repair pathway. So far, *RNF113A* has only been implicated in one family with intellectual disability and trichothiodystrophy (TTD), which is a group of rare autosomal recessive disorders that variably affect a wide range of organs derived from the neuroectoderm. In this report, we describe two related fetuses with a phenotype with dysmorphic features, microcephaly, callosal and cerebellar malformations, absent testis and micropenis. WES was performed on one of the fetuses and both parents, and led to the identification of a nonsense variant in the highly conserved *RNF113A* gene (c.901 C>T, p.Q301*). Sanger sequencing analysis confirmed the variant was present in both fetuses and inherited from the mother.Our report provides two new cases with a *RNF113A* variant. This furnishes more consistent evidence of a role of *RNF113A* in neurodevelopment with functional studies that suggest a role in DNA repair pathway.

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E-P11.69
Two novel ROR2 mutations in Robinow syndrome

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Introduction: Robinow syndrome is a genetically heterogeneous skeletal dysplasia, is due to mutations in the ROR2 (MIM: 268310), WNT5A (MIM: 180700), DVL1 (MIM: 616331) and DVL3 (MIM: 616894) genes. ROR2 mutations exhibit autosomal recessive inheritance pattern whereas WNT5A, DVL1, DVL3 mutations segregate as autosomal dominant trait. Robinow syndrome is characterized by skeletal abnormalities encompass mesomelic or acromesomelic limb shortening, brachydactyly, distinctive facial features and genital hypoplasia.

Materials and Methods: We diagnosed three cases (two of whom siblings) of Robinow syndrome with typical clinical features. All coding exons and the flanking intronic regions of the ROR2 gene amplified by PCR and performed bidirectional direct sequencing. Variations were detected by comparing the reference sequences and interpreted by in silico predictive programs.

Results: Two novel mutations [(c.1386+1G>A), (c.784_785insCGAGGTGCTGGAGAGCG, p. D262Afs*189)] were identified in ROR2 gene. The first mutation (c.1386+1G>A) is located in the spice donor site of the intron 8 that affects the protein structure by damage splicing. The other mutation is occurred in exon 6 by inserted 17 base that lead to disrupt the amino acid sequence. In silico analysis predicted both of the novel mutations to be pathogenic.

Conclusions: This study revealed the first ROR2 gene splice-site mutation and expanded the database for ROR2 mutations in patients with Robinow syndrome.

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E-P11.70
Extending the critical regions for mutations in the noncoding gene RNU4ATAC in another patient with Roifman syndrom

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Introduction: Roifman Syndrome (RFMN; OMIM #616651) is an autosomal recessive inherited disease characterized by growth retardation, humoral immunodeficiency, intellectual deficit, distinctive facial features and in some individuals retinal dystrophy. This syndrome is caused by compound heterozygosity in the gene *RNU4ATAC*, coding for the snRNA U4atac, which is an essential component for minor intron splicing.

Material and Methods: Here we describe a female five year old patient presenting with growth retardation, recurrent infections, organ anomalies and developmental delay. The patient´s DNA was tested before by microarray analysis. No causative results could be detected. Due to genetic heterogeneity a trio analysis was performed on clinical exome sequencing using the Illumina TruSight One sequencing panel. The focus of our analysis lay on genes associated with multiple congenital anomalies and / or developmental delay.

Results and Conclusions: Compound heterozygosity of two SNVs could be detected in the gene *RNU4ATAC*. The paternally inherited substitution n.13C>T in the stem II region had been described previously, while the maternally inherited substitution n.116A>C affecting a highly conserved nucleotide in *RNU4ATAC* had not been described before. We propose that these variants are the likely cause of the patient's phenotype, thereby extending the spectrum of pathogenic variants in *RNU4ATAC*.

1

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E-P11.71
Three Cases of Rubinstein-Taybi Syndrome with Novel *de Novo* cAMP binding protein-BP (CREBBP) Mutations

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Introduction: Rubinstein-Taybi syndrome (RSTS; OMIM #180849) is a rare autosomal dominant genetic condition with a prevalence of 1 in 125,000-720,000. RSTS is characterized by broad thumbs and halluces, facial dysmorphism, short stature and a variable degree of intellectual disability. Pathogenic variants in CREBBP and EP300 genes cause RSTS in 50-60% and 5-8% of cases, respectively. The majority of the cases occur as a result of de novo heterozygous mutations.

Materials and Methods: We suspected RSTS based on particular clinical findings and dysmorphic features of the patients. Patient DNA samples were extracted from the peripheral blood, followed by CREBBP whole gene sequencing.

Results: In one patient two novel heterozygous missense variants were detected: c. 5034C>G and c.5047C>T. In other two patients, we found novel heterozygous frameshift mutations: c.3250_3250+3delAGTA and c.4599-4599delT, respectively. In silico analyses predicted these mutations as deleterious/disease-causing alterations. Parental mutation analyses of patients were normal and confirmed the de novo occurrence of the variants.

Conclusions: We have identified four novel CREBBP variants in three patients demonstrating clinical features of RSTS.

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E-P11.72

Co-occurrence of *SHOX* region (Xp22.3) rearrangements and 15q25.2 duplication in a girl with short stature, genital defects and bone anomalies

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The presence of multiple genomic unbalances in the same patients co-operate to cause complex phenotypes. Here we describe a 9-year old girl, diagnosed with short stature with growth hormone deficiency and precocious puberty. She had agenesis of right tibia and fibula, a supernumerary digit of the left foot, dorsal scoliosis, short neck, upturned nose, hypotelorism and arterial hypertension. MRI showed uterus didelphys with double vagina. An array CGH analysis revealed the presence of two distinct duplications flanking the *SHOX* gene within the PAR1 region (Xp22.3) and an additional duplication of 1.6-2.5 Mb at 15q25.2. The duplicated region on 15q25.2 contains 13 genes, some of them have been associated to well defined disorders but not included in the clinical features observed in our patient. The parents were not available to establish the origin of the duplications. The extreme short stature of the girl might be the result of the co-occurrence of growth hormone deficiency and the rearrangements in the *SHOX* region. Molecular defects of *SHOX* and/or its regulatory region explain 60-80% of cases with Leri-Weill dyschondrosteosis and 7-15% of cases with idiopathic short stature and are associated to hypoplasia/aplasia of the ulna and fibula. Few previously described patients carrying duplication at 15q25.2 presented some clinical features resembling those observed in our patient such as anomalies of the foot digits, hypertension, and short neck. In conclusion, the complex phenotype of our patient is likely to represent the result of a never described co-occurrence of unbalances in the *SHOX* region and on 15q25.2.

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E-P11.73

IGF2 mutation causing Silver-Russell syndrome in an Aboriginal Australian girl

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Introduction: Silver-Russell Syndrome (SRS OMIM 180860) is a rare disorder characterised by severe intrauterine and postnatal growth retardation with additional clinical criteria. It remains a clinical diagnosis with a molecular cause identifiable in 60-70%. We present a 4-year-old Aboriginal Australian girl with an SRS phenotype who was extensively investigated prior to referral to our Undiagnosed disease program through which *IGF2* was investigated as candidate gene based on clinical interdisciplinary panel assessment.

Materials and Methods: Trio whole genome sequencing was performed by Genome.One on extracted DNA on the Illumina HiSeq X instruments using a TruSeq Nano DNA Library Preparation kit. Trusight One sequencing was applied simultaneously. cDNA sequencing was performed to investigate variant function detected thereby.

Results: Simultaneously, both platforms identified a heterozygous intronic substitution c.157+3A>C. This was absent from in-house and population databases and Alamut predicted splice site abolition. Sanger sequencing confirmed de novo status, cDNA studies show skipping of the exon containing the initiation codon. Cumulatively, studies confirmed the variant was on the paternal allele and pathogenic.

Conclusion: We identified a novel *IGF2* splice site variant causing a SRS phenotype in an Australian Aboriginal girl. We compare and contrast clinical findings with reported patients to increase the *IGF2* knowledge base, including noting the frequency of cardiac, neurodevelopmental and limb phenotypes across different ethnic groups;

and to promote engagement of other Australian Aboriginal families in genomic medicine.

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Cri-du-chat syndrome mimics Silver-Russell syndrome depending on the size of the deletion

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Introduction: Silver-Russell Syndrome (SRS) is a rare growth-related genetic disorder in which patients show intrauterine (IUGR) and postnatal growth retardation (PNGR), relative macrocephaly at birth, body asymmetry, facial dysmorphic features and feeding difficulties and/or low BMI. Approximately 50% of the clinically diagnosed SRS patients present alterations at 11p15.5, mainly hypomethylation at *H19/IGF2*:IG-DMR, whereas 10% of them show UPD(7)mat. For the remaining 40% negative SRS patients, molecular karyotyping is advised.

Materials and Methods: We present a 22 months old girl with clinical suspicion of SRS (IUGR, PNGR, prominent forehead, triangular face, psychomotor delay, transient neonatal hypoglycaemia, mild hypotonia and single umbilical artery). She also presented corpus callosum hypoplasia, mild ventriculomegaly, Arnold-Chiari malformation and high-pitched voice. Methylation and CNVs at chromosomes 11 and 7 were studied by MS-MLPA (MRC Holland, ME030-C3 and ME032-A1, respectively). Molecular karyotyping was performed (aCGH, Agilent 400K).

Results: After no alterations were detected by MS-MLPA at the analysed DMRs, the aCGH identified a deletion at 5p15.33p15.2 (arr[hg19] 5p15.33p15.2(25,942-11,644,645) x1). Deletions at 5p are commonly associated with Cri-du-chat syndrome (CdC).

Conclusions: The absence of some CdC features in our patient could be due to the fact that their critical responsible regions are not included within the identified deletion. The common clinical aspects of CdC and SRS (impaired growth, developmental and intellectual delay) alongside the macrocephaly and the triangular face, typical of SRS, led to the initial clinical misinterpretation.

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Duplication of 15q24.1q26.2 and terminal deletion of 15q26.2q26.3 in a female child with severe growth impairment

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The patient was the first child of unrelated 27-year-old parents. Her mother suffered from immunoglobulin A (IgA) nephropathy but the rest of her family history was unremarkable. Pregnancy was complicated by foetal growth restriction and breech presentation. She was born at 37 weeks and 5 days of gestation by caesarean section. Her birthweight was 1647 g (-3.2 SD), length was 40.5 cm (-3.1 SD), and head circumference was 30.0 cm (-2.1 SD). She had facial features characteristic of micrognathia. G-Banded karyotyping identified interstitial duplication of chromosome 15q with the karyotype 46,XX,dup(15)(q22-24q26.2). At the age of 4 years and 7 months, the child was referred to the Tokyo Metropolitan Kita Medical and Rehabilitation Center for the Disabled for her developmental disability. Her height of 82.1 cm (-5.3 SD) suggested a growth factor deficiency. To identify the precise chromosomal breakpoint, we performed single nucleotide polymorphism (SNP) array analysis at the Tokyo Medical University Hospital. The SNP array identified arr[hg19] 15q24.1q26.2(73,922,340-98,178,660)×3, 15q26.2q26.3 (98,179,896-102,429,112)×1. It revealed that not only did she have duplication of 15q24.1q26.2 as previously reported, but also terminal deletion of 15q26.2q26.3. In the deleted region 15q26.2q26.3, the insulin-like growth factor-1 receptor gene (*IGF1R*) was involved. This deletion was thought to be the cause of her severe growth impairment.

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SNParray diagnostic yield in children with intellectual disability with and without malformations in a Romanian population

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Microarray analysis currently represents one of the first line genetic assessment tool for patients with intellectual disability, dysmorphic features and/or malformations.

Aim: We evaluated the diagnostic yield of SNParray in a Romanian population with this phenotype spectrum.

Method: We used HumanCyto SNP BeadChip Kit (Ilumina). Data analysis was performed using DECIPHER (Wellcome Sanger Institute) and UCSC Genome Browser (University of California).

Results: The cohort included 78 paediatric patients (39 females; 6+/-5,6 years) presenting intellectual disability with or without cranio-facial dysmorphism, malformations or developmental. In some cases, karyotyping and fluorescent in situ hybridization were used to identify chromosomal rearrangements. A pathogenic copy number variation was identified in 24,3%(19/78) of cases (microdeletion or microduplication syndromes) and 11.5% had possibly pathogenic or uncertain variants. The pathogenic copy number variants included 15 microdeletions (chromosomes 1,3,11,12,15,17,18 and X) and 4 microduplications (chromosomes 15,13,16). The patogenic variants confirmed the syndromic phenotypes. For uncertain variants (all duplications chromosomes 1,5,21), parental SNPmicroarray testing had been used to elucidate inheritance. Average size of deletion was 12,6Mb(range 1,1-40Mb).Uncertain variants had size range between 1-5,4Mb. CONCLUSSION The diagnostic yield in our cohort was relatively good, however it included cases that were already diagnosed using karyotype and SNParray provided additional accuracy regarding size and genes involved in the copy number variant. Nonetheless, a good clinical selection is crucial for efficiency of genetic testing. Unsolved cases require further molecular testing.

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E-P11.77
A newborn with Sotos syndrome and renal polycystosis

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We report the case of a female newborn with Sotos syndrome and renal polycystosis.Before birth,renal polycystosis was already diagnosed on echographies.At birth, she was hypotonic and dysmorphic and she had big difficulties to drink.Cardiac echography has shown an aortic stenosis Renal polycystosis was confirmed.CGH-array has revealed a deletion of 1863,4 kb in the region 5q35.2-q35.3 involving the NSD1 gene,hereby confirming Sotos syndrome.The search for mutation in PKD1,PKD2,ARPKD and in a panel of genes for renal polycystosis was negative. We will discuss the possibility of implications of other genes deleted and the particular characteristics of Sotos syndrome in a newborn with renal polycystosis.

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E-P11.78
As a rare case of chromosomal anomaly, 49, XXXXY syndrome

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49, XXXXY syndrome is characterized by the presence of three extra X chromosomes, and this syndrome is described as the rarest sex chromosomal aneuploidy syndrome. Classical findings include mental retardation, hypogonadism, and radioulnar synostosis. There are a limited number of cases reported in the literature. It is sometimes referred to as a variant of Klinefelter syndrome, but differs from Klinefelter syndrome in many ways and is more severe. Severe varying degrees of learning difficulty and mental rotation are encountered; Some patients have deteriorated with age. The patient was referred to our polyclinic with dyslexia, speech delayed, micropenis, microorchidism, atypical face appearance, hypertelorism, broad nasal root, short philtrum, radioulnar stenosis. The patient's mother and father were not relatives. There is no evidence in the patient's family history. Chromosome analysis was applied to the patient and his parents. Because the patient's karyotype was determined as 49, XXXXY, the patient's findings were identified as 49, XXXXY syndrome. parents' karyotype results were normal. Our case was carrying the cardinal clinical findings of the 49, XXXY syndrome, which had been previously reported. In our patient, mental retardation has been shown to worsen with age, as seen in some patients with this syndrome.

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E-P11.79
The multiple faces of TMEM231

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Pathogenic variants in the *TMEM231* gene (OMIM 614949) have been reported to cause a spectrum of phenotypes, including Joubert syndrome, Meckel-Gruber syndrome and Oro-facial-digital 3 syndrome. We evaluated three sibs from a consanguineous family affected with a spectrum of phenotypes which included retinal degeneration, intellectual disability and renal abnormalities. All three had early childhood onset of retinal degeneration, however, clinical features were different between them to a great extent. The proband's facial gestalt included down-slanting palpebral fissures, convex and high nasal bridge, short philtrum and prominent incisors, slender palms, truncal obesity, short stature and hypogonadism, raised a suspicion of Cohen syndrome. His older sister had severe developmental delay and profound intellectual disability, and his younger one had borderline learning disabilities and nephronophthisis diagnosed in adolescence. Using exome sequencing we identified a homozygous c.511T>G (p.L171V) novel variant in the *TMEM231* gene (GeneBank accession number NM_001077418.2) which is fully segregated in the family. To date, at least 13 different pathogenic variants of *TMEM231* have been reported. These variants are spread throughout the gene and associated with several partially overlapping, yet distinct phenotypes, with no clear genotype-phenotype correlation. Moreover, we report significant phenotypic heterogeneity within the same family. The finding expands the phenotypes that can be caused by pathogenic variants *TMEM231*, which should be considered in learning disabilities associated with retinal degeneration and Cohen syndrome-like phenotype, and strongly suggest the existence of additional environmental and/or genetic factors which modify the phenotype induced by *TMEM231* variants.

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E-P11.80
Long-time follow-up and hearing impairment in four patients with Treacher Collins syndrome

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Treacher Collins syndrome is a rare disorder of craniofacial development that affects first and second branchial arches. The estimated incidence is 1/50,000 live births. Mutations in *TCOF1*(5q32) (78%-93%) and *POLR1C* or *POLR1D* (8%) cause the disease. 40%-50% of individuals have conductive hearing loss attributed to malformation of the ossicles and hypoplasia of the middle ear cavities. We describe 4 patients to discuss the frequency of clinical features and audiological data, illustrate evocative/particular features and evolution in time. All cases have been sporadic and result from uneventful pregnancies. Case 1 (male, 10 years): IUGR, microcephaly, downslanting palpebral fissures, hypoplastic zygomatic arches, dental malposition, cleft palate, deformed/lowset ears, bilateral auricular tags, mild-severe bilateral conductive hearing loss; epilepsy; hiatal hernia. Case 2 (male, 11 years): triangular face, mildly asymmetric, downslanting palpebral fissures, lower eyelids coloboma, hypoplastic zygomatic arches, micro-retrognathia, dysplastic ears, bilateral severe conductive hearing loss. Case 3 (male, 15 years): triangular face, mildly asymmetric, downslanting palpebral fissures, lower eyelids coloboma, hypoplastic zygomatic arches, deformed/lowset ears, severe bilateral conductive hearing loss. Case 4 (male, 18 years): microcephaly, triangular face, downslanting palpebral fissures, everted lower eyelids, sparse lashes, dental malposition; tympanosclerosis; aortic coarctation; epilepsy. Apart of epilepsy (worse in time), the clinical picture has been constant. Clinical features at different ages will be illustrated. In conclusion, we present the evolution in time and the importance of a multidisciplinary approach in the managament of the patient. Early detection of hearing loss in these patients is of great importance in order to reabilitate hearing impairment.

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E-P11.81
A Novel*TCOF1*Gene Mutation In A Turkish Family With Treacher Collins Syndrome

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Introduction: Treacher Collins Syndrome (TCS), also known as Mandibulofacial Dysostosis is an autosomal dominant disorder of craniofacial development and presents variable expressivity and mainly caused by mutations in the *TCOF1* gene. The major features are commonly include otologic, ophthalmic and dental malformations and midface hypoplasia, micrognathia, microtia, conductive hearing loss and cleft palate can be seen. Treacher Collins Syndrome occurs with an incidence of 1 in 50000 live births. Phenotypes caused by mutations in the gene *POLR1D* and *POLR1C* have similar clinical features with different inheritance patterns.

Materials and Methods: A 32 years old man applied to our clinic with characteristic facial dysmorphism with bilateral and symmetrical hypoplasia of the malar bones. He had antimongoloid slant of the palpebral fissures, ear abnormalities and hearing loss. Both of his son were died because of the respiratory distress and they were both have malar hypoplasia, malformation of auricle, hearing loss, downslanting palpebral fissures and partial absence of lower eyelashes. His father also has mild antimongoloid slant of the palpebral fissures. For mutation analysis, the coding region of *TCOF1* gene was sequenced.

Results: The molecular analysis showed the presence of heterozygous mutation, c.4394C>T / p.Ser1465Leu in exon 25 of the *TCOF1* gene in the proband and his father. To our knowledge, the mutation has never been reported.

Conclusion: Both variable expressivity and reduced penetrance must be considered in and also it is very important that; healthcare providers must be careful not to miss the mild findings especially of autosomal dominant diseases.

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E-P11.82

A novel mutation in the TCOF1 gene in a patient with Treacher Collins syndrome

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Treacher Collins syndrome (TCS), is a rare autosomal dominant congenital disorder characterized by various craniofacial malformations. The estimated incidence is 1/50000 live births. There are bilaterally symmetric anomalies of the structure within the first and second branchial arches. The characteristic facial dysmorphism includes bilateral and symmetrical hypoplasia of the malar bones and mandible. This syndrome most commonly results from the TCOF1 gene mutations. Here we report a five-year-old female patient who has a syndromic appearance and hearing loss. The patient had various facial dysmorphic features including downward-slanting eyes, malar hypoplasia, micrognathia, fishlike mouth with a high arched palate and absent lower eyelid eyelashes. There were also malformed bilateral pinnae and left ear microtia. According to the clinical features, we suspect from TCS and sequence analysis of TCOF1 gene was performed. A heterozygous new mutation c.1722_1731delCATCCTCCAG in exon 12 of the TCOF1 gene was detected. It has been determined that this mutation is pathogenic according to the in silico prediction tools. The current study further expands the TCOF1 mutation spectrum.

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E-P11.83

Rare case of triphalangeal thumb-polysyndactyly syndrome in combination with congenital heart disease

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<META NAME="author" CONTENT="Ольга Седенко">

Introduction: Triphalangeal thumb-polysyndactyly syndrome (TPT-PS) caused by *LMBR1* mutations is a well-defined autosomal dominant disorder not commonly associated with heart defects. There is only one report on a patient with TPT-PS and Tetralogy of Fallot, however, harboring an additional 22q11.21 deletion. Here we present a 3-month-old girl with prenatally established double outlet right ventricle (DORV). Additionally, the patient had asymmetric preaxial, postaxial polydactyly with TPT-PS (4-5 digits). Similar phenotype of upper and lower limbs was reported along five generations in 9 affected family members with no history of congenital heart disease.

Materials and Methods: The investigation was approved by the local ethical committee. Informed consent was obtained from patient's parents. Genetic analysis was performed using Sanger sequencing and array-based comparative genomic hybridization (CGH-array) with Agilent 60K platform.

Results: Target sequencing of the *TBX5* gene to exclude Holt-Oram syndrome, *NKX 2-5*, intron 5 of the *LMBR1* did not reveal any known pathogenic variants/variants of unknown significance. Array-CGH allowed to identify the only duplication at 7q36.3 region (~ 277 kb), encompassing *LMBR1* gene.

Conclusions: *LMBR1* is a causative gene leading to TPT-PS but it has not previously been associated with DORV.

Further analysis of possible association of *LMBR1* duplication with cardiac defects should include sequencing of broad spectrum of genes (GDF1, CFC1, ZFPM2) or even exome sequencing approach to exclude possible combination of two independent genetic abnormalities as a cause of the combined malformation pathologies.

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E-P11.84
Homozygote *TTN* gene variant associated with lethal congenital contracture syndrome

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Introduction: Pathogenic variants in the *TTN* gene have been reported to cause various cardiomyopathies and a range of skeletal muscle diseases, collectively known as titinopathies.

Materials and Methods: Eight affected individuals presented with lethal congenital multiple contractures belong to a large family of Moslem Bedouin origin, living in the northern part of Israel, were studied. Exonic sequences from three patients' DNA samples were enriched with the SureSelect Human All Exon 50 Mb V.5 Kit

Results: A homozygous c.36122delC (p. P12041Lfs*20) variant in exon 167 of the *TTN* gene, which is only expressed in the fetal IC isoform, was detected in all patients.

Conclusions: Our findings provide evidence for the causative role of *TTN* gene in the severe lethal form of arthrogryposis multiplex congenita. The finding expands the phenotypes that can be caused by pathogenic variants in *TTN* gene, which should be considered in lethal congenital contracture syndromes, arthrogryposis multiplex congenita, congenital myopathies and hydrops fetalis.

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E-P11.86
Synemin expression profile during tissue-specific differentiation of mesenchymal stromal cells confirms its possible involvement in heart-hand syndrome

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Introduction: «Heart-hand» syndromes represent a group of rare congenital conditions where patients in addition to cardiac pathology demonstrate various abnormalities of limb skeleton. To date, genetic basis of such phenotypes is only partly characterized. In the present study, we report on a 30-year-old woman with a clinical picture closely resembling ulnar-mammary syndrome. The patient was hospitalized due to frequent episodes of non-sustained ventricular tachycardia and presented with symmetrical fifth finger anomalies, mammary gland hypoplasia with inverted nipples, specific face features, squint and abnormal teeth. Here we aimed to extend knowledge on molecular mechanisms underlying the complex congenital disorder.

Materials and Methods: Genomic screening was carried out by whole-exome sequencing (WES), Sanger sequencing and array-based comparative genome hybridization.

Results: No genetic defects were revealed in *TBX3*, the only gene associated with ulnar-mammary syndrome to date, as well as in other genes responsible for common heart-hand syndromes. Based on WES data analysis, we took notice of a novel missense variant in *SYNM* gene (exon 1, p.A58V), encoding an intermediate filament protein synemin. Experimental cell study confirmed high expression of synemin in mesenchymal stromal cells (MSCs) during tissue-specific differentiation, thus, pointing to a possible role of synemin in the congenital malformations.

Conclusions: Here we present a nontypical clinical case of ulnar-mammary syndrome with no association with *TBX3* mutation. We suggest that the genetic variant in

SYNM could contribute to the complex phenotype, but further comprehensive functional studies are required.

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E-P11.87

Combined Partial Trisomy 7q and Partial Monosomy 21q in a 6 Year Old Male Patient Phenotypic and Genotypic Findings

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Proband is 6 years old male with dysmorphic features and examined by psychomotor retardation pre-diagnosis. The boy was born after uneventful pregnancy at term with normal growth parameters. In infancy, he had feeding difficulties. His mother noticed that eye colors were different from each other when he was 1 month old. The patient underwent surgery for inguinal hernia at 14 months and 2 years of age. When he was 1 year old underwent surgery due to in his right eye tension. He was able to sit without support and hold his head after 1 year of age. He can walk holding on to one place at the moment. At 5 years of age, additional features were noted including brachycephaly, beak nose structure, low set ears, upslanting palpebral fissure,retro micrognatia, ortognati, short filtrum. Cytogenetic analysis of cultured lymphocytes revealed a 46,XY,der(21)(pter→q22::?) karyotype. Mother was normal, father's karyotype 46,XY,t(7;21)(q32;q22). Array-CGH was performed oligonucleotide array and showed 7q33q36.3 gain, 21q22.2q22.3 loss. Very often fetuses with complete monosomy 21 die before or shortly after birth; by contrast, cases with partial deletion of chromosome 21, which occur more often, have better survival expectancy and are heterogeneous regarding their phenotypic severity. Although duplications of the chromosome 7q are well known, these are rare and their presence usually results in the apparition of multiple congenital abnormalities and cognitive deficits. We present the first report of a patient with 21q22 deletion and concomitant 7q33 duplication detected by CGH array and associated with dysmorphism, psychomotor retardation.

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E-P11.88

Novel bi-allelic *POMGNT1* mutations detected by whole exome sequencing causing dystroglycanopathy in a family of Cypriot descent

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Congenital muscular dystrophies represent a clinically and genetically heterogeneous group of inherited muscle disorders. Affected infants typically present with hypotonia and poor spontaneous movements. Progressive weakness, joint contractures, spinal deformities and respiratory compromise may affect quality of life and life span. Cognitive impairment, structural brain and/or ocular abnormalities and seizures are found almost exclusively in dystroglycanopathies.

A cohort of undiagnosed Cypriot patients with multiple congenital malformations was selected for whole exome sequencing (WES) following extensive diagnostic workup. We report on a non-consanguineous family with two male siblings, born following uneventful pregnancies. They presented with a congenital muscular dystrophy phenotype with features including infantile hypotonia, hyperCkemia early hydrocephalus, severe developmental delay, epilepsy, strabismus and cataracts. Family history was non-contributory. WES was performed on the Illumina NextSeq500 platform using the TruSeq DNA exome library kit. Bioinformatics analysis was carried out using an in-house pipeline.

Two novel compound heterozygous variants were identified in *POMGNT1* and confirmed by Sanger sequencing. These included a nonsense mutation (p.Glu102*) in exon 4 and a missense variant (p.Arg129Trp) in exon 5 which substitutes a conserved aminoacid within the interleukin-like epithelial to mesenchymal tissue (EMT) domain of *POMGNT1*. Both variants are classified as rare and were not identified in online databases. *In-silico* tools predicted pathogenicity. Parental carrier status was confirmed.

In conclusion, two affected siblings carrying novel, predicted pathogenic, bi-allelic *POMGNT1* variants were identified. Other *POMGNT1* mutations have been associated with autosomal recessive dystroglycanopathies exhibiting similar phenotypes. Further investigations to measure the expression of the defective protein are ongoing.

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E-P11.89

Williams-Beuren syndrome: clinical variability in a Nord-Eastern Romanian cohort

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Williams-Beuren (WS) syndrome (7q11.23 microdeletion), is a syndrome with wide clinical variability. We have studied 22 patients diagnosed with WS and followed-up for a long time (average period 7 years) in Iasi Medical Genetics Center, aiming to describe clinical variability and the changing picture in time. All cases were confirmed using MLPA follow-up kit. We identified cardiovascular disease in 18/22 patients (72.22% supravalvar aortic stenosis), characteristic dysmorphic faces in 19/22, intellectual disability in 19/22 (5 severe, 9 moderate, 5 mild) and connective tissue defects in 16 cases (40.95% presented hernia).

Less common features included: pre- and early post-natal growth deficiency (18/22), friendly personality (16/22), specific cognitive profile (13/22), strabismus and dental anomalies (9/22), gastro-intestinal disturbances (9/22), obesity (6/22), hypercalcemia (3/22) and hypothyroidism (1/22). Short stature and increased sensitivity to sound were found in only 7 and 5 patients, and only 1 case presented hearing loss and enuresis. Cryptorchidism, as well as renal defects were identified in 4 cases, anesthesia accidents in 2 and diabetes and hypoplastic abdominal aorta in one.We didn't detect non-social anxiety, specific phobias or early puberty. Other features identified in our patients include: brachydactyly (5/22), autism spectrum disorder (4/22), dysmenorrhea (2), hypogonadism (3/22), scoliosis (2/22), marked microcephaly and psoriazis (1/22) and ischemic stroke (1/22). The changing phenotype in time will be illustrated for cases with long term follow-up. In conclusion, this study emphasizes the wide phenotypic variety from one case to another, and within the same patient at different ages.

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E-P11.90

Xia-Gibbs syndrome presenting with craniosynostosis, tethered cord and Chiari I malformation

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Xia-Gibbs syndrome (MIM 615829), a multisystem disorder first described in 2014, is caused by heterozygous variants (including nonsense variants) in *AHDC1* that encodes the AT hook DNA-binding motif containing 1 (AHDC1) protein. All 15 individuals reported to date have had global development delay. Other frequent features include hypotonia, facial dysmorphism, laryngomalacia, short stature, microcephaly, obstructive sleep apnea, hernias and corpus callosum hypoplasia.

Studies in mice and drosophila suggest that *AHDC1* is an epigenetic regulator of embryonic brain development. Pathogenic *AHDC1* variants may act via a dominant-negative mechanism.

We present a 5 year old boy with moderate developmental delay, a large omphalocele, metopic craniosynostosis, short stature, dysmorphic features, hypotonia and chronic snoring. Snoring improved after bilateral adenotonsillectomy. Brain and spinal cord imaging revealed metopic craniosynostosis and a tethered cord, both of which were treated surgically, as well as Chiari I malformation and a shortened corpus callosum. The only variant of interest detected on whole exome trio sequencing was de novo heterozygous NM_001029882.3(*AHDC1)* c.2772del p.(Arg925Glufs*).

Metopic and bicoronal craniosynostosis was reported previously in a single individual with Xia-Gibbs syndrome. Our case suggests a tentative extension of the phenotype to include craniosynostosis, tethered cord, Chiari I malformation and omphalocele.

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E-P12 Cancer genetics

E-P12.10

Impact of MTHFR C677T variant on the susceptibility of acute leukemia in Tunisia

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Introduction: The association between the 5,10-methylenetetrahydrofolate reductase (MTHFR) variants; mainly the C677T; and the risk of acute leukemia (AL) was largely investigated and enrolled in a meta-analysis. However, the results were inconsistent.

The aim of this study was to investigate the relationship between the C677T variant of the *MTHFR* gene, and susceptibility to acute leukemia in a cohort of Tunisian patients with AL.

Materials and Methods: A case-control study was conducted among 37 patients with AL and 35 controls. Genotyping of *MTHFR* C677T was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: The frequency of *MTHFR* 677T was significantly different between AL patients and controls (21.6% and 0% respectively), independent of the gender [$p = 0.58$; OR=1.56 (0.31-7.78)]. The risk of AL with the 677T allele carrier was elevated by 2.2 times ($p = 0.001$).

Conclusions: The present result highlights the impact of *MTHFR* C677T variant on the susceptibility of acute leukemia in Tunisian patients. Thus, *MTHFR* C677T polymorphism may be a promising AL biomarker and further study with larger numbers of participants worldwide are required before definitive conclusions can be drawn.

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E-P12.15

Apoptotic Effects of *Myrtus communis* L. Essential Oil on A549 Cells

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Introduction: *Myrtus communis* L. (MC) is an important aromatic and medicinal plant. Understanding of the molecular mechanism of apoptotic cell death in cancer studies has great importance. In this study, we aimed to investigate whether the essential oils of MC induce apoptosis on A549 cells, and if it is induced, which pathway leads the cells to apoptosis.

Materials and Methods: A549 cells were exposed to various concentrations from 31.25 to 2500 μg/ml of the essential oils. The cytotoxicity was measured by MTT assay and IC_{50} values were determined. Then, the essential oil was administered at the concentrations of 312.5 μg/ml for 24 hours. The expression levels of caspase 3, 8 and 9, Bcl-2 and P21 genes were evaluated by qRT-PCR method.

Results: Our results showed that 625 μg/ml and above concentrations of the essential oils significantly suppressed cell viability. Expression levels of caspase 3 and 9 genes which are mediators of intrinsic pathway were significantly increased at a concentration of 312.5 μg/ml of essential oils ($p = 0.0129$ and $p = 0.0180$ respectively). However, when the expression levels of the caspase-8 gene, an important mediator of extrinsic apoptosis pathway, were compared with the control group, no difference was found. It was found that, the p21 and the anti-apoptotic Bcl-2 genes were not different when compared with the control group.

Conclusions: As a result, the essential oils of MC induce apoptosis through the intrinsic pathway. In this regard, we think they may be new hope for non-cytotoxic drug researches in the treatment of cancer.

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E-P12.16

Evaluation of a novel BAP1 mutation found in a family with high cancer burden

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BAP1 tumour predisposition syndrome (BAP1TPS) is a recently described cancer syndrome associated with various types of cancer such as melanoma (eye and skin), malignant mesothelioma, renal clear cell carcinoma, basal cell carcinoma and atypical Spitz tumour (benign). *BAP1* (BRCA1-associated protein 1) is a tumour suppressor gene that encoding ubiquitin carboxyl-terminal hydrolase. This enzyme regulates cell growth, cellular differentiation, transcription and DNA repair. Mutation on both alleles can lead to rapid and uncontrolled cell growth. The germline mutation in *BAP1* gene is inherited in an autosomal dominant manner.

The proband in a family with high burden of renal clear cell carcinoma and one case with mesothelioma, was

offered gene panel testing. The gene panel used consisted of 19 renal cancer associated genes. The only suspicious variant detected, was a missense variant in the *BAP1* gene, c.325G>A, p.(Gly109Arg). This variant has not been published as a BAP1TPS predisposing variant before, and because of lacking information it was classified as a variant of uncertain clinical significance. The family history and further analyses of blood and tumor samples from family members, and how this contributed in the reclassification of the variant will be presented.

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E-P12.17

Screening of Polish patients for novel BRCA1/2 mutations as underlying factors of ovarian/breast cancer

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Introduction: A strong genetic predisposition towards breast and ovarian cancers is frequently tied to mutations in *BRCA1* and *BRCA2*. Women with pathogenic mutations in either *BRCA1* or *BRCA2* have about five times higher risk of breast cancer and ten to thirty times higher risk of ovarian cancer, but not all changes confer the same risks. While the most common genetic determinants are known, in high-risk patients novel loss-of-function mutations can contribute a significant risk.

Materials and Methods: Screening of 806 samples over the 2016-2017 period has confirmed the prevalence of founder mutations: c.5266dupC, c.4035delA, c.181T>G, c.3700_3704delGTAAA and c.68_69delAG in BRCA1 (NM_007294.3). In cases lacking the above, full Next Generation Sequencing was performed using IonTorrent S5 platform. The DNA library was prepared using AmpliSeq BRCA1/BRCA2 Panel (ThermoFisher Scientific). The presence of pathogenic mutations was confirmed by Sanger sequencing on GA3500 (Applied Biosystems).

Results: In one case, (63y.o. female with breast cancer and suspected ovarian cancer) a novel BRCA2:c.3215T>A (p.L1072*) mutation was detected. The likely loss-of-function follows due to heavily truncated protein product. Additionally, we detected several rare but previously described mutations with predicted deleterious impact.

Conclusions: In high-risk cases with negative screening results for common founder mutations, there is a strong cause for extended analysis of whole BRCA1/BRCA2 genes. Such an approach allows to detect new/atypical underlying changes in ca. 22% of affected patients. Routine sequencing of key oncogenes is thus validated as a tool of choice for informed therapies in breast/ovarian cancer treatment.

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E-P12.18

Detection of BRCA1/BRCA 2 gene mutations in ovarian cancer by next- generation sequencing

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Introduction: Mutations in BRCA1 and BRCA2 genes lead to an increased risk of developing breast or ovarian cancer. In high-grade serous ovarian cancers (HGSOC) the frequency of BRCA1/2 germline and somatic mutations ranged from 18 % to 26% (Alsop et al. 2012; Ledermann et al. 2011). Identification of mutations in BRCA1/2 genes could facilitate targeted therapy with selective PARP1 inhibitor.

Materials and Methods: Genomic DNA was isolated from 101 HGSOC patients who underwent surgical resection between 2013 and 2016 (47 microdissected FFPE tumor tissues and 54 peripheral blood samples used when quality of DNA from FFPE tissue was not satisfactory. BRCA 1/2 mutation analysis was performed by targeted next-generation sequencing (NGS), using the ONCOMINE™ BRCA1/BRCA2 Research Assay (Life Technologies, USA) covering all exons and intron-exon boundaries. Multiplex PCR, DNA library preparation and enrichment were performed according to manufacturer`s instructions. Sequencing was carried out on Personal Genome Machine (PGM) sequencer (Ion Torrent, USA). The sequence data, including alignment to the hg19 reference genome and variant calling was done using Torrent Suite Software v4.6 and Ion Reporter™ Softwere (Life Technologies, USA).

Results: Pathogenic BRCA 1/2 mutations were found in 28,7% HGSOC patients (29/101); in 17 FFPE and 12 blood

samples. 26 mutations were located in BRCA1 gene and 3 in BRCA2 gena. The mutations were SNV and short indel introducing frameshift or stop codon. No large deletions or insertions were detected in our study group.

Conclusion: Detection of BRCA1/2 mutations in FFPE tumor tissues using NGS method is feasible.

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E-P12.20

Genetic susceptibility to cancer: is it time to delegate testing prescription to oncologists and breast/gynecological surgeons? A pilot project

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Introduction: Cancer genetics clinics struggle to cope with steady increases in referrals. Indeed, awareness among the general public and medical professionals is rising, tumour profiling is becoming commonplace with its incident discovery of cancer susceptibility mutations, and PARP-inhibitors might soon be approved for indications other than relapsing serous ovarian carcinoma. One must therefore explore alternative modes of genetic counseling and novel approaches to testing, a task all the more challenging in France where only cancer geneticists (CG) have been prescribing genetic tests. **Objectives**. We are starting a pilot project involving CG, oncologists and breast/gynecological surgeons (O+OBGS) from a Paris University Hospital and its affiliated suburban partner, and studying the feasibility and acceptability of a partnership allowing O+OBGS to prescribe genetic tests.

Methods: All patients with breast/ovarian cancer will receive a family questionnaire. O+OBGS will evaluate whether testing is indicated using a modified Manchester-Scoring-System (MSS3). They will explain the principles of genetic susceptibility to cancer, give an explanatory leaflet, obtain consent and prescribe *BRCA1/2, PALB2*, and *RAD51C/D* analysis. CG will receive a medical report, the family questionnaire and a copy of the MSS3. If they suspect Li-Fraumeni or think a multigene panel is indicated, they will ask to see the patient face-to-face. CG will return all results during an individual consultation. Outcomes will be compared to patients seen exclusively by CG. **Perspectives**. We will present preliminary observations at ESHG-2018. Our novel approach should allow us to cope with referral increases, and free up time for consultations requiring real cancer genetics expertise.

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E-P12.22

Characterizing of five common BCR-ABL kinase domain mutations (T315I, F359V, E255V, E255K, Y253H) in Bulgarian patients with chronic myeloid leukemia preliminary results

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Introduction: Chronic myeloid leukemia (CML) arises from the fusion of the BCR and the ABL1 genes due to reciprocal chromosome translocation forming the Philadelphia Chromosome (Ph). Mutations in the Bcr-Abl kinase domain may cause, or contribute to, resistance to tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia patients. The aim of our study is to detect five common mutations (T315I, F359V, E255V, E255K, Y253H) in the ABL1 kinase domain (harboring on acquired) in patients with CML.

Materials and Methods: The study was performed on 30 patients with CML. We used Allele-specific amplification to detect the mutations in the ABL1 kinase domain.

Results: In our study T315I mutation was detected in one patient and in the same patient E255K mutation was present as well. We did not detect E255V, F359V and Y253H mutations in the patients. The patient is a 79 years old male, diagnosed in CML - chronic phase in April 2017. The patient was treated with Nilotinib, followed by Dasatinib. Not even hematological response has been achieved so far.

Conclusions: The patient with mutations in ABL kinase domain showed treatment failure based on 2013 ELN recommendations for management of CML. One of the study limitations was limited number of samples. Research on larger number of patients is suggested to obtain more reliable results. Identification of ABL kinase domain mutations may be used as a proper and useful method for improving therapeutic strategies, avoiding delay in

treatment and excessive expenditure in CML patients with tyrosine kinase inhibitors resistance.

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E-P12.24

Fibronectin type III domain containing 5(FNDC5) gene expression level in colorectal cancer cell lines

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Metabolic activities are altered in cancer cells relative to normal cells, which provide a selective advantage during tumorigenesis, and support the acquisition and maintenance of malignant properties. Precisely how metabolism becomes reprogrammed in cancer cells, whose functions or malignant properties are enabled by these activities, help to exploit metabolic changes for suitable therapeutic benefit. On the other hand, reprogrammed metabolism refers to the ability of cancer cells supporting the increased energy request due to continuous growth, rapid proliferation, and other characteristics typical of neoplastic cells. FNDC5/Irisin, a novel hormone in the regulation of energy balance, is involved in metabolic homeostasis. Energy imbalance were shown to be associated with colorectal cancer. Therefore, FNDC5 expression level was assessed in two colorectal cancer cell lines i.e. Caco-2, HT29 and human fibroblast as a control group by quantitative real-time PCR. Our results indicated that FNDC5 expression level is increased significantly in HT29 colorectal cancer cell line compare to Caco-2 colorectal cancer cell line and control group. A positive association with FNDC5 expression and metastatic colorectal cell line(HT29) suggested the potential role of this gene as one of energy metabolism regulator in metastatic colorectal cancer cells.

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E-P12.25

The effect of helicobacter pylori cagA gene on autophagy pathway in human gastric cancer cells

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Lung cancer is the leading cancer killer of both men and women throughout the world. Notably, KRAS accounts for 90% of RAS mutations in lung adenocarcinomas. Recent literature has reported a strong association between KRAS mutation and survival in non-small cell lung cancer and approximately 97% of KRAS mutations in NSCLC involve codons 12 or 13. Molecular mechanisms that are mediating tumor's initiation, invasion, metastases, recurrence and resistance to the therapy in lung cancers remains largely unknown. One of the target sequences of miRNA Let-7 complementary site within the 3'untranslated region of KRAS gene (named is 3'-UTR LCS6 sequence). LCS6 mutation may lead to metastases, development of cancer, relapse and treatment resistance in many cancers. In this project, it was aimed to carry out the effect of LCS6 mutation on metastatic functions and AKT and ERK pathways, by using lung cancer A549, NCI-H441 cells and BEAS-2B (control) cell carrying pLenti-KRAS-CDSmLCS6n, pLenti-KRAS-CDSm-LCS6m plasmids. The results showed that G12V and LCS6 mutations have significant different effects among A549 and NCI-H441 cell groups. the mutation of LSC6 region increases the cell migration and proliferation in NCI-H441 cells, while in A549 cells a slight decrease occurred in migration and proliferation levels. Both G12V and LCS6 mutations increase the cell invasion level in A549 and NCI-H441 cells. As for the target genes, both Western and qPCR results showed that G12V and LCS6 mutations induced PI3K/AKT/mTOR and RAF/MEK/ERK pathways in A549 and NCI-H441 cells, induce these pathways were higher in A549 cells.

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E-P12.26

Human malignant glioma: implementation of the adjunctive therapy by transferring the CRISPR/AsCpf1 system-induced tumor suppressor genes insertion and oncogenic suppression of Bcl2L12 via the CRISPR/ddAsCpf1 system for in vivo engineering of chromosome 19 q-arm

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Glioblastoma multiform (GBM) is the most prevalent intracranial primary brain tumor in adults which can cause a strikingly complex process by altering various genes and molecular biomarkers. *These research indicated that chromosome 19q harbors many tumor suppressor genes that are integral to glioblastoma carcinogenesis*. These genes localized the common region of deletion to the distal long

arm, 19q13.2-13.4. EML2 is one of the tumor suppressor genes that is situated on 19q13.32. Also, this loci can be considerably methylated in GBMs by CpG island promoter hypermethylation. Interestingly, miR-330 encoding gene is lies inside the intron of EML2, and this miRNA also acts as oncosuppressor-miR in glioblastomas. Importantly, miR-519d encoding gene which is situated on 19q13.42 also downregulated in GBMs. Moreover, NOP53 (also designated GLTSCR2/PICT-1) is localized within the distinguished 1.4 Mb tumor suppressive region of chromosome 19q13.33. Additionally, Bcl2L12, a protein belonging to the Bcl-2 protein family which is located on 19q13.33, is upregulated in glioblastoma multiform (GBM) and plays a role in tumor cell development and tumor cell resistance to apoptosis. Our hypothesis is that a programmable CRISPR/AsCpf1-based genome engineering strategy can be used to attach these sizeable chromosomal fragments of tumor suppressor genes as an essential part of the chromosome 19 q-arm with the aim of chromosomal treatment. Besides, we can simultaneously employ CRISPR-ddAsCpf1 system for specific Inhibition of Bcl2L12 as an oncogene in glioma cells. In this research cancer-derived exosomes-associated scAAV9 is utilized as a convenient delivery platform of CRISPR/AsCpf1 and CRISPR/ddAsCpf1 systems to GBM cells.

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E-P12.27

Molecular alterations in Bulgarian patients with Glioblastoma multiforme

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Introduction: Central nervous system tumors are characterized by invasive nature and difficulties to resect surgically all neoplastic cells with frequent relapses. Glioblastoma multiforme (GBM), classified as World Health Organization (WHO) grade IV infiltrative glioma, is the most common primary brain tumor in adults and the most lethal subtype of gliomas. The molecular heterogeneity in gliomas is extremely complex. There are several genes involved in critical signaling pathways like receptor tyrosine kinase (RTK), p53 pathway and retinoblastoma (RB) pathway. Somatic copy number variations in these genes are supposed to contribute to the glioblastoma development and prognosis. In the present study, we examined the presence of genetic aberrations in a small group of Bulgarian patients.

Materials and Methods: Fresh brain biopsy tissues were analyzed with multiplex ligation - dependent probe amplification (MLPA).

Results: Simultaneous genetic alterations were found: EGFR *(epidermal growth factor receptor)* amplification, CDKN2A (cyclin dependent kinase inhibitor 2A) deletion and TP53 (tumor protein p53) deletion.

Conclusion: This is an initial study on signaling regulators genetic aberrations in Bulgarian glioblastoma patients. Detected genetic variations are correlated with *differentiation* of primary and secondary gliomas and disease outcome but more analyses are needed to assess their prognostic role. The study was partially supported by Medical University Sofia, Bulgaria

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E-P12.28

Does Theranekron affect the pathway of apoptosis & endoplasmic reticulum stress in head-neck cancer cell

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Introduction: *Tarantula cubensis* venom (*Theranekron*) is used as a homeopathic medicine. Many therapeutic effects of Theranekron, such as antitumor, necrotizing action and wound healing have been demonstrated in clinical studies. ER stress has a role in development of inflammation and cancer. Therefore, ER stress has been suggested as a promising target for tumor cell death and cancer therapy. Effectiveness of theranekron as an anti-cancer chemical has been demonstrated in limited human cancer cells. However, a study related to the effect of theranekron on head cancer is not available in the literature. Therefore, in this study, we aimed to investigate the effect of theranekron on ER stress and apoptosis on HNSCC.

Materials and Methods: Effective doses of theranekron (80 µl) in the study were determined by MTT analysis. Cells were treated with 80 µl theranekron for 6 hours. Total RNA was isolated using TRIzol reagent. Synthesis of cDNA from the total RNA was carried out using Transcriptor High Fidelity cDNA Synthesis kit. Expression levels of genes (GRP78, CHOP, EDEM1) associated with endoplasmic reticulum stress and genes (P53, BAX, BCL-2) associated with apoptosis were analyzed by RT-qPCR. Fold changes were calculated by the ΔCT method. The statistical significances were analyzed applying two-tailed student's t-test and analysis of variance (ANOVA).

Results: As a result, we found a difference in the expression levels of genes associated with ER stress and apoptosis in theranekron-treated HNSCC cells compared to the untreated group. This suggests that theranekron effects the pathways associated with ER stress and apoptosis.

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E-P12.30

Assessment of JAK2 V617F mutation in Tunisian patients with essential thrombocythemia

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Introduction: Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by thrombocytosis with a tendency to thrombosis and hemorrhage. Acquired single point mutation in the JAK2, the V617F, has been described as a frequent genetic event in patients with ET (approximately 50-60 %).

In this study, we have investigated the JAK2V617F mutation in Tunisian patients with ET, to establish the correlation between phenotype and genotype.

Materials and Methods: Mutation screening for JAK2 V617F in patients was performed by allele-specific PCR in 12 newly diagnosed ET patients in Tunisia. Clinical and biological data were recorded.

Results: JAK2 V617F mutation was found in 2 patients with an overall frequency of 16.7%.A particular history of thrombosis was recorded.

Conclusions: Despite the limitation of our study, the JAK2 V617F mutation seems to be not frequent in Tunisian patients with ET. Moreover, genotyping of patients suspected of a myeloproliferative neoplasm for JAK2 v617F would be useful to assess the risk of complications. Larger studies are mandatory.

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E-P12.31

Effects of kras gene lcs6 mutation on metastasis pathways in human lung cancer

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Lung cancer is the leading cancer killer of both men and women throughout the world. Notably, KRAS accounts for 90% of RAS mutations in lung adenocarcinomas. Recent literature has reported a strong association between KRAS mutation and survival in non-small cell lung cancer and approximately 97% of KRAS mutations in NSCLC involve codons 12 or 13. Molecular mechanisms that are mediating tumor's initiation, invasion, metastases, recurrence and resistance to the therapy in lung cancers remains largely unknown. One of the target sequences of miRNA Let-7 complementary site within the 3'untranslated region of KRAS gene (named is 3'-UTR LCS6 sequence). LCS6 mutation may lead to metastases, development of cancer, relapse and treatment resistance in many cancers. In this project, it was aimed to carry out the effect of LCS6 mutation on metastatic functions and AKT and ERK pathways, by using lung cancer A549, NCI-H441 cells and BEAS-2B (control) cell carrying pLenti-KRAS-CDSmLCS6n, pLenti-KRAS-CDSm-LCS6m plasmids. The results showed that G12V and LCS6 mutations have significant different effects among A549 and NCI-H441 cell groups. the mutation of LSC6 region increases the cell migration and proliferation in NCI-H441 cells, while in A549 cells a slight decrease occurred in migration and proliferation levels. Both G12V and LCS6 mutations increase the cell invasion level in A549 and NCI-H441 cells. As for the target genes, both Western and qPCR results showed that G12V and LCS6 mutations induced PI3K/AKT/mTOR and RAF/MEK/ERK pathways in A549 and NCI-H441 cells, induce these pathways were higher in A549 cells.

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A PHENOTYPIC CONTINUUM FROM LYNCH SYNDROME TO CMMRD

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Colorectal cancer (CRC) is extremely rare below the age of 25 years even in Lynch syndrome (LS), which is caused by heterozygous germline mutations in the mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*). On the other hand, biallelic germline mutations in one of these MMR genes are responsible for the Constitutional Mismatch Repair Deficiency (CMMRD) syndrome characterized by an early-onset CRC with a high childhood cancer risk.

We present a very early-onset CRC in a 12 year-old boy belonging to a LS family with a germline pathogenic variant in the *MSH2* gene. His CRC showed MSH2/MSH6 protein-complex loss, microsatellite instability, and the second-hit mutation in *MSH2*. Due to his early-onset a CMMRD was suspected, but the absence of another detected germline alteration in the MMR genes together with the results obtained in the gMSI /(ev)MSI and the methylation tolerance assays[1] discarded the CMMRD diagnosis.

Our hypothesis is that another germline variant occurring in the affected child could contribute in addition with the causative *MSH2* variant to his early-onset CRC. Whole-exome-sequencing and trio analysis were carried out.

Preliminary results showed no genetic alterations in actionable genes related to hereditary colorectal cancer syndromes or in genes involved in DNA repair pathways (NER, BER, HR). Further analyses are still undertaking.

These data support the existence of a severe and early-onset LS phenotype mimicking CMMRD. Although the genetic mechanisms underlying this clinical continuum are largely unknown, we will discuss possible scenarios of a postulated genetic continuum.

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E-P12.34
Detection of mitochondrialDNA copy numbers in the plasma of ovarian cancer patients

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Introduction: Mitochondria are the key organelles in the energy production, cell proliferation, and apoptosis. Copy number variations were connected to different pathological conditions. It was suggested that it is contributing factor to different cancer development. We decided to measure the mtDNA copy numbers in the plasma in ovarian cancer patients and healthy controls.

Materials and Methods: We involved 24 healthy controls (average age 54.00±14.57 years) and 24 ovarian cancer patients (average age 61.33±12.72 years) in the study. Blood was drawn into EDTA tubes and plasma was separated by centrifugations. MtDNA copy numbers were determined by Human Mitochondrial DNA (mtDNA) Monitoring Primer Set kit (Takara, Japan) with real-time PCR.

Results: The mtDNA copy number was 48±17.34 in the healthy control group. We divided the ovarian cancer patients group according to their FIGO classification, the copy numbers were the followings FIGO I. 32±8.38; FIGO III. 33±5.66; FIGO IV. 42±18.87. There is not significant difference in the plasma cell-free mtDNA copy numbers.

Conclusion: We determined the cell-free mtDNA copy numbers in the plasma of ovarian cancer patients and healthy controls and did not found significant difference. We plan to extend the study to exosome encapsulated mtDNA measurements.

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E-P12.36
Expression quantitative trait loci analysis of nasopharyngeal carcinoma

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The discovery of expression quantitative trait loci (eQTL) of specific tissue can help to reveal genetic component of complex diseases. To identify eQTLs responsible for

nasopharyngeal carcinoma risk behind the loci identified in genome-wide association studies (GWAS), we conducted eQTL analyses using 25 tumor and 5 adjacent normal tissues. The *cis*- and *trans*-eQTLs were identified by integrating whole genome genotyping data with RNA-Seq gene expression data. After analysis, there were 18,375 SNP-mRNA eQTL pairs passed P < 1.00E-05 criteria. Then the eQTLs were combined with NPC GWAS loci and found *HLA-B*, *HLA-DQ* and *TNFRSF19* were with putative NPC susceptibility eQTLs.

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E-P12.39

Investigation of the effect of theranekron on endoplasmic reticulum stress in human pancreatic cell line BXPC-3

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Introduction: *Tarantula cubensis* venom (*Theranekron*®) is used as a homeopathic medicine which has shown anti-tumor effects in veterinary medicine. The aim of this study was to assess effect of *Tarantula cubensis* on apoptotic cell death of human cancer cell lines. ER stress has also a key role in development of inflammation, obesity and cancer. Thus, ER stress has been suggested as a promising target for tumor cell death and cancer therapy. Effectiveness of theranekron as an anti-cancer chemical has been demonstrated in various human cancers. However, a study related to the effect of theranekron on pancreas cancer is not available in the literature. Therefore, in this study, we aimed to investigate the effect of tunicamycin-induced ER stress on BXPC-3 cell line.

Materials and Methods: Effective doses of theranekron (2μg/ml) in the study were determined by MTT analysis. Cells were treated with 2μg/ml theranekron for 6 hours. Total RNA was isolated using TRIzol reagent. Synthesis of cDNA from the total RNA was carried out using Transcriptor High Fidelity cDNA Synthesis kit. Expression levels of genes (GRP78, CHOP, EDEM1, ATF4, ATF6, XBP1) associated with endoplasmic reticulum stress were analyzed by RT-qPCR. Fold changes were calculated by the ΔCT method. The statistical significances were analyzed applying two-tailed student's t-test and analysis of variance (ANOVA).

Results: As a result, We found a difference in the expression levels of ER stress related genes in theranekron-treated BXPC-3 cells compared to the untreated group. This suggests that theranekron effects the pathways associated with ER stress.

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E-P12.40

Can Theranekron be an agent for inducing apoptosis and ER stress in human pancreatic cells

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Introduction: *Tarantula cubensis* venom (*Theranekron*) is used as a homeopathic medicine. Many therapeutic effects of Theranekron, such as antitumor, necrotizing action and wound healing have been demonstrated in clinical studies. ER stress has a role in development of inflammation and cancer. Thus, ER stress has been suggested as a promising target for tumor cell death and cancer therapy. Effectiveness of theranekron as an anti-cancer chemical has been demonstrated in limited human cancer cells. However, a study related to the effect of theranekron on pancreas cancer is not available in the literature. Therefore, in this study, we aimed to investigate the effect of theranekron on ER stress and apoptosis on PANC-1.

Materials and Methods: Effective doses of theranekron (10 μl) in the study were determined by MTT analysis. Cells were treated with 10 μl theranekron for 4 hours. Total RNA was isolated using TRIzol reagent. Synthesis of cDNA from the total RNA was carried out using Transcriptor High Fidelity cDNA Synthesis kit. Expression levels of genes (GRP78, CHOP, EDEM1) associated with endoplasmic reticulum stress and genes (P53, BAX, BCL-2) associated with apoptosis were analyzed by RT-qPCR. Fold changes were calculated by the ΔCT method. The statistical significances were analyzed applying two-tailed student's t-test and analysis of variance (ANOVA).

Results: As a result, we found a difference in the expression levels of genes associated with ER stress and apoptosis in theranekron-treated PANC-1 cells compared to the untreated group. This suggests that theranekron effects the pathways associated with ER stress and apoptosis.

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E-P12.41

Does Tarantula cubensis venom have antipoliferative & endoplasmic reticulum stress inhibitor activity on skin cancer cells?

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Tarantula cubensis venom (*Theranekron*) is used as a homeopathic medicine which has shown anti-tumor effects in veterinary medicine. ER stress has also a key role in development of inflammation, obesity and cancer. Thus, ER stress has been suggested as a promising target for tumor cell death and cancer therapy. Effectiveness of theranekron as an anti-cancer chemical has been demonstrated in limited human cancer cells. However, a study related to the effect of theranekron on pancreas cancer is not available in the literature. Therefore, in this study, we aimed to investigate the effect of theranekron on ER stress and apoptosis on SK-MEL-30 cell line.

Materials and Methods: Effective doses of theranekron (20 µl) in the study were determined by MTT analysis. Cells were treated with 20 µl theranekron for 24 hours. Total RNA was isolated using TRIzol reagent. Synthesis of cDNA from the total RNA was carried out using Transcriptor High Fidelity cDNA Synthesis kit. Expression levels of genes (GRP78, CHOP, EDEM1, ATF4, ATF6, XBP1) associated with endoplasmic reticulum stress and genes (P53, BAX, BCL-2, BAD) associated with apoptosis were analyzed by RT-qPCR. Fold changes were calculated by the ΔCT method. The statistical significances were analyzed applying two-tailed student's t-test and analysis of variance (ANOVA).

Results: As a result, we found a difference in the expression levels of genes associated with ER stress and apoptosis in theranekron-treated SK-MEL-30 cells compared to the untreated group. This suggests that theranekron effects the pathways associated with ER stress and apoptosis.

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E-P12.43
Association of rs944289 polymorphism and differentiated thyroid cancer: a pilot study in Thai population

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Introduction: Many single nucleotide polymorphisms (SNP) have been shown to be associated with differentiated thyroid cancer (DTC) in Caucasoid and Asian populations. Among those reported, the rs944289 seems to had a significant association in Asians. Here we investigated the role of rs944289 as a pilot study in Thai population.

Materials and Methods: 179 patients with confirmed thyroid cancer and 98 healthy individuals as control group were enrolled. Genotypes were identified using PCR-RFLP methods. Results were analyzed by SPSS.

Results: The risk T allele of rs944289 polymorphism was not associated with risk of thyroid cancer, with odds ratio of 0.97 (95% CI 0.68-1.38) in Thai population. These result revealed that the rs944289 polymorphism is not a main risk factor for thyroid cancer in Thai.

Conclusions: The rs944289 SNP, however, may not play a primary role in the development of DTC in Thai population. Further studies would include more samples and extend to other reported variants to create a profile polymorphisms for thyroid cancer susceptibility in Thai population.

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E-P13 Basic mechanisms in molecular and cytogenetics

E-P13.02
Different origin of chromosome 15 CNVs

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Introduction: The 15q11-q13 region contains many low copy repeats, is well known for its genomic instability and is substrate for CNVs formation. CNVs may be detected by aCGH and NGS. But these methods provide information only about copy number variations (deletion, duplication, triplication).

Materials and Methods: We studied CNVs of chromosome 15 detected by aCGH in 4 cases and by NGS - in one case. FISH analysis was performed on interphase and metaphase chromosomes with CEP and LSI DNA-probes on chromosome 15.

Results: In two cases, the copy number gains (dup15q11.2q11.3 and dup15q11.2) were caused by interstitial duplication detected by FISH analyses.

In two other cases (triplication15q11.2q13/duplication15q13.2q13.3 and duplication15q11-q13) FISH-analysis showed inv dup 15 supernumerary marker chromosome.

In one case, with the deletion of 15q11.2q13.3 (8,7Mb) an unbalanced translocation - der(13)t(13;15)(q11.1,13.3) was found. The genomic imbalance detected by CMA was due to an unbalanced segregation product of a paternal balanced translocation.

Conclusions: Our data demonstrate that although aCGH and NGS may be should be the first-tier test for clinical diagnosis of genomic imbalance, but only chromosome analysis or FISH provides the chromosomal structural information associated with these copy number changes. Some of these copy number changes are associated with additional chromosomal structural rearrangements that can only be defined by FISH analysis with targeted DNA-probes. Identification of chromosomal structural rearrangements is indispensable for genetic counseling of the family.

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E-P13.03

A rarely seen 47,XXY/46,XX mosaicism of Klinefelter syndrome: Case report

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A rarely seen 47,XXY/46,XX mosaicism of Klinefelter syndrome: Case report

Introduction: Klinefelter syndrome is one of the most common human sex chromosome anomalies. Males with Klinefelter syndrome may have one or more extra copy of the X chromosome in each cell (47,XXY/48,XXXY/49, XXXXY) or may have the extra X chromosome in only some of their cells. This condition is described as mosaic (47,XXY/46,XY). 47,XXY/46,XX mosaicism of Klinefelter syndrome is very rare and aproximately 10 cases have been reported so far in the literature.

Clinical case: We report here a case of a mosaic 47, XXY/46,XX infertile male in whom referred to our department with infertility history for 5 years. His sperm analysis revealed azoospermia. The cytogenetic analysis with GTG-banding karyotype lymphocytes from peripheral blood samples showed 47,XXY[57]/46,XX[43]. Also FISH analysis with X centromere probe confirmed the mosaicism.

Conclusion: We have demonstrated 47,XXY/46,XX mosaicism of Klinefelter syndrome which is extremely rare genetic disease and therefore we thought to contribute to the literature with the present study.

Keywords: 47,XXY/46,XX mosaicism, Klinefelter syndrome, rare disorders.

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E-P13.06

Molecular cytogenetic survey of alterations to cell cycle pathway in neuropsychiatric diseases

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Introduction: Chromosome and genomic rearrangements are a frequent cause of abnormal brain functioning resulting in neuropsychiatric diseases. Despite a wide variety of available data, specific biological processes underlying abnormal neurodevelopment remain to be unmasked. Here, we have used molecular cytogenetic data to analyze specific pathways leading to neuropsychiatric diseases in children.

Materials and Methods: Conventional and molecular karyotyping was used to evaluate 427 patients with idiopathic intellectual disability, autism, epilepsy, and congenital malformations. An original bioinformatic technique based on candidate process prioritization (Iourov et al., 2014; Yurov et al., 2017) was applied to pathway enrichment among these patients.

Results: Molecular cytogenetic analysis has shown CNVs relevant to a pathway functional change in 397 children (93%). The highest enrichment rates (gene percentage among all the prioritized candidate genes/processes) were found for the following pathways: cell cycle (4.68%), MAPK signaling (6.56%), pathways in cancer (11.94%), DNA replication (1.64%). All the children exhibiting the genomic variations altering genes involved in these pathways were found to present with chromosome instability (mosaic aneuploidy, chromosome breaks/fragility, non-specific structural chromosomal rearrangements), whereas the remainder was not demonstrating definable chromosome instability.

Conclusions: Alterations to pathways associated with genome/chromosome stability maintenance through the cell cycle were shown to be involved in the pathogenesis of neuropsychiatric diseases. The consequences of the alterations were found to be detectable at chromosomal level. These data supports a previously proposed hypothesis describing two-/multiple hits to genome stability as a possible mechanism of neuropsychiatric diseases (Iourov et al., 2013). Supported by RSF (14-35-00060).

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E-P13.08DR

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The frequency of genetic disorders among people reaching adulthood is estimated to be equal 8%. Due to the clinical and genetic heterogeneity of diseases, molecular diagnosis can be very challenging. Whole-exome sequencing (WES) have revolutionized the field of genomics by allowing a rapid and cost-effective diagnosis.

Here, we present data on the first 172 probands referred to us for genetic evaluation by WES. Patients presented with a range of phenotypes suggesting potential genetic causes.

The molecular diagnostic rate observed in our study is 43%.

This yield is higher than the positive rates of other genetic tests, such as karyotype analysis (5 to 15%), chromosomal microarray analysis (15 to 20%), and Sanger sequencing for single genes.

WES provided a high diagnosis in people with deafness, renal dysfunction, leukodystrophies and neuromuscular disorders; suggesting that these groups of patients are particularly good candidates for genetic evaluation by WES.

Undiagnosed cases may present large rearrangements or mutations that are located in non-coding regions, such as regulatory or deep intronic regions, which can not be detected by WES. Furthermore, technical limitations such as poor coverage can also interfere with WES success.

In conclusion, the use of WES to analyze 172 clinical cases yielded a diagnosis in 43% of these cases, which supports the use of WES as a diagnostic test for patients with nonspecific or unusual disease presentations of possible genetic cause and for patients with clinical diagnoses of heterogeneous genetic conditions.

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E-P13.10

The routes of r(13) instability in induced pluripotent stem cells

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Introduction: The mitotic inheritance of ring chromosomes in induced pluripotent stem cells (iPSCs) has acquired special interest after report, that iPSCs can eliminate ring chromosome and restore a normal karyotype via uniparental disomy (Bershteyn et al., 2014).

Materials and Methods: We obtained 3 iPSC lines from fibroblasts of patient with r(13) by using LeGO lentiviral vectors, coding OCT4, SOX2, KLF4 and C-MYC transcription factors. Cytogenetic analysis was performed using G-banding and FISH.

Results: Fibroblasts were mosaic for variable level of r (13), monosomy 13 and 46,XY,-13,+mar cells during cultivation. iTAF6-6 iPSC line karyotype was 46,XY,r(13) in 80% and 45,XY,-13 in 20% of cells at passage 13 (P13). iTAF6-13 line had 95% of 46,XY,-13,+mar cells at P11, whereas the ring chromosome was not registered at all. Perhaps, this clone originated from a cell with the marker chromosome or fragmentation of r(13) took place during reprogramming or at early passages. In iTAF6-4 line the proportion of cells with r(13) significantly decreased from 18% to 5%, at the same time the proportion of cells with marker chromosome significantly increased from 73% to 86% (at P5 and P12 respectively) with a small number of monosomic cells.

Conclusions: r(13) was instable during reprogramming or at early passages in iPSCs. However, spontaneous correction of the 46,XY,r(13) karyotype to normal has not been registered in any of the cell lines. This study was supported by Russian Science Foundation, grant 16-15-10231.

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E-P13.13

Chromosomal damage in Individuals with radiofrequency eposure as Measured by Personal Exposimeter

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Introduction: Communication and information technologies rely on electromagnetic radiations and the increased dependency on these technologies has resulted in an insidious build-up of radiofrequency electromagnetic field (RF-EMF) in the environment. The RF radiation exposure may pose to be health-hazardous and, therefore, requires assessment.

Materials and Methods: In the present study, chromosomal damage was assessed using the Buccal Micronucleus Cytome (BMCyt) assay as a function of radiation exposure after Institutional Ethics Committee clearance of the study and written voluntary informed consent from the participants. For this, 24 hour-personal exposimeter measurements (PEM) were recorded for unrelated 60 healthy adults (40 cases residing in the vicinity of mobile phone base stations since their installation and 20 controls residing in areas with no base stations).

Results: The PEM in cases (149.28±8.98 mV/m) revealed significantly higher ($p = 0.000$) electric field strength compared to the recorded value (80.40±0.30 mV/m) in controls. The cases (n=40; 23-90 years) and the controls (n=20; 19-65 years) matched for alcohol drinking, smoking habits, and mobile and cordless phone usage. The frequencies of micronuclei (1.86X, $p = 0.007$), nuclear buds (2.95X, $p = 0.002$) and cell death parameter (condensed chromatin cells) were significantly (1.75X, $p = 0.007$) elevated in cases compared to that in controls probably as a function of radiofrequency radiation exposure in the absence of other exposure(s).

Conclusions: The long- term exposure to low intensity electromagnetic microwaves as emitted continuously by mobile phone base station may provoke ill health effects which may further lead to cancer development. However, studies on a larger sample size are required for more insights.

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E-P13.14
fishing with the ring

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Ring Chromosomes are rare cytogenetic abnormalities. Teloremic deletion or fusion, disturbing chromosome stability, was the most incriminated mechanisms.We report in this work, the case of a newborn (patient 1) presenting a polymalformative syndrome (severe dysmorphic syndrome and a cardiopathy) and a 16 years-old boy (patient 2) with scoliosis, Madelung deformity and aortic stenosis.Patients underwent a standard R-band blood karyotype and fluorescence *in-situ* hybridization (FISH).Karyotype showed in patient 2 the presence of a chromosome mosaic with a ring 7 chromosome present in 28% of mitoses and a derivative chromosome 7 (der (7)) present in 72% of mitoses. Der (7) is a duplicated in 7q terminal. Patient 2 presents a chromosomal mosaic associating a monosomy X and a ring Y chromosome. FISH confirmed the presence of the ring Y chromosme in 90% of the analyzed nuclei. This Y ring chromosome contains the *SRY* gene Instability of ring chromosomes during mitotic divisions explains their mosaic distribution. Phenotypes associated with ring chromosomes are variable depending on the nature of the ring chromosome, the mosaic rate and their genes content. Ring 7 and Y chromosomes are chromosome abnormalities. During the cell cycles, by an exchange effect between sister chromatids, ring chromosomes can undergo breaks, duplication,⋯ These phenomena can lead to the formation of derivative chromosomes associated with the ring.The haploinsufficiency of *SHOX* gene in ring Y chromosome is responsible for 70% of Léri-Weill dyschondrosteosis. Characterization by CGH array of these ring chromosomes is in progress and may explain their formation mechanism

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E-P13.15
Impact of diet, household income and stress on telomere length in a cohort of Colombian schoolchildren

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Introduction: Mean telomere length (TL) has been associated with aging, cancer, diet and stress. In order to assess the effects of diet and stress on TL, and minimise the effect of age, the current study assessed school-age children with a comprehensive phenotyping approach.

Materials and Methods: Relative telomere length ratios were determined in 375 subjects (age 9-14 years). Subjects were recruited within the ACFIES (Association between Cardiorespiratory Fitness, Muscular Strength and Body Composition with Metabolic Risk Factors in Colombian Children) study. Phenotypes were determined using standard tests and an extensive questionnaire. A composite stress score was derived by adding together the number of serious life events for each subject. Statistical analysis was carried out using SPSSv25 (IBM Inc., USA).

Results: Consumption of 31 different foods was not significantly associated with TL ($p>0.05$). Uncorrected associations were observed for dairy ($p = 0.014$), fast food ($p = 0.01$) and fruit juice ($p = 0.049$). Household income was positively associated with consumption of dairy ($p = 0.00005$) and fruit juice ($p = 0.03$) but not fast food ($p>0.05$). It was negatively associated with a composite stress score ($p = 0.00003$). In this study, neither household income nor stress were associated with TL ($p>0.05$).

Conclusions: Interestingly, TL appears to be positively associated with consumption of dairy products, possibly due to the beneficial effects of increasing micronutrients such as calcium in the diet for these children. The lack of association with household income and stress may be due to the relatively small cohort size, or their young age so that long-term effects are yet to manifest.

E.D. Kidd: None. **A. Walley:** None. **J. Buxton:** None. **J. Le Page-Pezet:** None. **D.D. Cohen:** None. **P. Lopez-Jaramillo:** None. **A.I.F. Blakemore:** None. **U.L. Fairbrother:** None.

E-P13.16
Gene expression profiles in response to *MCM4* transcription silencing

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The *MCM4* gene belong to the key proteins involved in the replication licensing in a cell cycle. The gene expression dysregulation was found in number of tumors. The aim of this study was to investigate the particularities of gene expression in cancer cells after *MCM4* gene silencing. Experiment was carried out on the HT-29 cell line. Profiling was made with the help of Affymetrix GeneChip 2.0. Data were normalized by SSTR Multi-Chip Analysis algorithm in TAC 4.0. The changes of *MCM4* RNA levels were determined through different time after siRNA transfection. Control cells were transfected with siCON1. A comparative analysis of siMCM4/siCON1 RNA samples showed that *MCM4* gene silencing leads to changes in 146 genes (gene-level p-value < 0,05). The most of identified genes are involved into regulation of cell cycle process, G1/S transition, DNA replication, anatomical structure development. In particular, the expression of *E2F7, Cdc6, CCNE2* cell-cycle genes were suppressed and expression of *CDK8* gene was increased. The *CDK8* is a part of mediator complex that allowed suggest activation of multiple transcription programs as response to gene silencing. Of significance is the increased expression of *TNFSF10* gene, which activates cancer cell death through the extrinsic apoptotic pathway. Both *TNFRSF10* (A/B) receptors constitutionally express into HT-29 cells. The data may suppose the activation of apoptosis through an external cell mechanism under *MCM4* silencing. Grant: № 0517 - 2017-0017, FASO Russia.

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E-P13.17
The two faces of *WTX* gene

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Abnormalities in WNT signaling are implicated in a broad range of developmental anomalies and in tumorigenesis. *WTX* is expressed during embryonic development and is somatically inactivated in 20-30% of Wilms tumors; germline mutations are also observed in both familial and sporadic forms of a X-linked dominant disorder, the

osteopathia-striata congenital with cranial sclerosis(OSCS). We reported two unrelated male probands: the first affected by OSCS (his mother present mild phenotype) and a second with familial Wilms tumor. Whole exome sequencing (WES) of both revealed two novel missense germinal mutations: c.401>G/T (OSCS) and c.477T>G (Wilms); additionally, WES on tumor tissue revealed a second hit in *CTNNB1* gene. Both germline mutations in males were found with a minor allele frequency <1% in Caucasian population. The histidine at 134 and the phenylalanine at 159 position in WTX were conserved in phylogenetic tree, thus suggesting a causative role. These variants are located in exon2 encoding an important Phosphatidylinositol(4,5)-bisphosphate binding domain to localize WTX to the plasma membrane. Most deleterious mutations in this domain correlate with fetal or neonatal lethality in affected males. We hypothesized that c.401G>T mutation is responsible of the "mild" male phenotype of OCSC, probably because of the change of a basic on hydrophobic amino acid (p.H134P) doesn't promote dramatically conformational change in the WTX. Furthermore, we reported in the second patient the first evidence of germinal predisposing mutation in *WTX* in familial Wilms tumor. We also hypothesized that gain-of-function or loss-of-function mutations at the same domain could cause very distinct clinical diseases.

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E-P14 New diagnostic approaches, technical aspects & quality control

E-P14.01
The effects of the different centrifugal gravities on the cell viability

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Cell culture is based on the principle of replicating cells under controlled conditions. Cell culture studies are an important part of today's popular research topics and are frequently used as models in vitro cancer research. The goal in cell and tissue culture is to keep the cells alive and to reproduce the cells and to freeze them for reusing when necessary. One of the most important points to note in experiments for cell culture studies is the amount of living cells used. While the qualification of the cell medium and the amount of the carbon dioxide and/or temperature of the incubator with CO_2 are include during passaging, the substance and/or method used are include among factors affecting cell viability during the freezing process. In addition to these factors, there is no definite information as to whether there is a relationship between centrifugation gradient and cell viability. We used eleven different cell lines, seven of which were monolayer (U-87MG, U-118MG, LN-18, PC-3, DU145, LNCaP, WI-38) and four of which were suspension (K-562, HL-60, CCFR-CEM, NCI-BL2171). We exposed cells to centrifugation with different time (5 and 10 min) and centrifugal gravity/rpm (600rpm/40g, 1200rpm/232g, 2400rpm/638g and 4800rpm/2550g) after passaging. Then, we evaluated dead and living cells by the Cedex XS analyzer. We found significant differs in cells viability for each cells after centrifugation process in parallel to time and centrifugal gravity. In conclusion, this study revealed differences in viability rates of cell lines in cell culture studies, depending on the centrifugation gradient.

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E-P14.02
Coverage analysis of targeted next generation sequence data among 204 primer immune deficiency patients

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Introduction: Next-generation sequencing is changing clinical routine of molecular diagnosis. As well as whole genome and exome sequencing targeted gene panels are getting increasingly used for diagnostic assays. Amplification imbalances is one of most common the limitations of multi-amplicon targeted sequencing.

Materials and Methods: We sequenced 204 primer immune deficiency diagnosed patients by community designed ready to use "Ampliseq Primary Immune Deficiency Research Panel v2" using 530 chip and Ion S5™ next-generation sequencer. Panel covers 264 gene and 5241 amplicons using two pools (2627 and 2614 amplicons respectively).

Results: Mean on target read was 97,86% and mean read depth was 340,98 where mean uniformity was 86,83%. We could not able to get any usable read from 42 of the 5241

amplicons (mean read count <0). Amplicon read count analysis revealed, 1844 and 3164 amplicons had at least one read count below threshold 10 and 20 read respectively. Amplification faults was not accumulated in specific genes and heterogeneously distributed.

Conclusions: Target based gene panels are helpful for genetic disorders where multi gene targets are needed to be sequenced. Because of analytic pipeline DNA enrichment is required. Biochemical nature of amplification is not a perfect process. While reporting multi gene panels clinicians have to consider the possibility of uncovered regions and using alternative methods for diagnosis. Here we share preliminary coverage analysis of primer immune deficiency panel sequencing data.

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E-P14.03

Large rearrangements in the CFTR gene: Data from patients of Greek origin

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Introduction: Cystic fibrosis (CF) (MIM#219700) is the second most common autosomal recessive disorder in Greece after thalassemias. The carrier frequency is estimated approximately at 4-5% of general population with an incidence of about 1 in 2500-3500 live births. Some patients, after sequencing the whole coding region and the exon - intron boundaries, have only one of the causative mutations identified. These patients carry on the other allele a large rearrangement that cannot be detected with conventional methods.

Materials and Methods: The study population was 45 patients, presenting with typical cystic fibrosis symptoms, with only one identified pathogenic mutation. We screened these patients for abnormal copy numbers (deletions and duplications) using MLPA® (SALSA MLPA P091 CFTR probemix, MRC Holland). Positive findings were confirmed using a custom made array CGH, specific for CFTR gene, platform 8x60K, G3 CGH+SNP microarray (Agilent Technologies, Santa Clara, CA, USA).

Results: A large rearrangement in the CFTR gene was detected in 28 out of 45 patients. Using a custom made aCGH we were able to map the exact breakpoints [GRCh37- 7q11.21].

Rearrangement	No of patients	Size (Kb)	Start	End
Dup10_12	10	41.499	117,186,717	117,228,215
Del2	6	7.822	117,138,471	117,146,292
Del4-8	5	10.695	117,169,806	117,180,500
Del2_22	2	130.63	117,139,946	117,270,575
Dup22	2	5.398	117,267,448	117,272,845
Del18_20	1	6.807	117,246,136	117,252,942
Del_upstream_9	1	76.283	115,913,429	117,196,552
Whole gene del	1	188.739	117,120,005	117,308,744

Conclusions: In Greece, as in other Mediterranean populations, CFTR mutations show great heterogeneity. In our study the frequency of large rearrangements is estimated to be approximately 2%. In conclusion we suggested that screening for such rearrangements should be included when screening patients with typical CF and when aiming to achieve a high detection rate.

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E-P14.04

Design and application of an expanded newborn screening NGS panel: first Argentinian experience

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Introduction: The aim of newborn screening (NBS) programs is to identify serious conditions that require early detection and urgent pre-symptomatic treatment in order to avert serious clinical harm. NBS in Argentina is mandatory for only 6 inborn errors of metabolism. NGS-based technologies allow laboratories to test multiple genes, thus enabling detection of several conditions simultaneously. Our aim was to develop the first expanded NBS panel in Argentina to offer a broad and rapid molecular diagnosis.

Materials and Methods: Guided by national and international NBS programs and recommendations from medical societies (ESHG, ACMG, AAP) for genetic testing in asymptomatic minors and interpretation of sequence variants, we selected severe childhood-onset disorders with available treatment that, if implemented early, would result in clinical benefits for the patient. An in-house pipeline for bioinformatic analysis was developed and a focused guideline for classification and report of variants was elaborated, as well as an algorithm for confirmatory tests.

Results: The current panel includes 96 genes for 50 diseases: 10 organic acid disorders, 8 fatty acid oxidation disorders, 8 lysosomal storage disorders, 6 aminoacid

disorders, 3 types of hyperammonemias, 2 immunodeficiencies, 2 hemoglobin disorders, 1 endocrine disorder and 10 miscelaneous conditions.

Conclusions: Currently, NBS by NGS should only be considered an add-on to traditional screening programs. However, it is a promising genetic test that may reduce false positives and negatives, shorten times required for diagnosis and enable genetic counseling, but many issues should be taken into account and properly discussed when designing the panel.

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E-P14.05
How to measure loss of chromosome Y

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Recent discoveries show that mosaic loss of chromosome Y (LOY) in leukocytes is associated with increased risks of cancer, Alzheimer's and cardiovascular disease. More than 15% of men older than 70 show some degree of LOY and these men survive on average only half as long as men without LOY. But how is LOY measured today and what would be the best method for the future?

Genome wide arrays are most commonly used in LOY-studies, but other methods have also been used: whole genome sequencing (WGS), gene targeted qPCR or droplet digital PCR (ddPCR). We have compared and evaluated arrays, WGS and ddPCR targeting a homologous gene located on the X and Y chromosome for LOY-studies. The cheap and easy ddPCR method gives very similar results as the expensive and precise WGS, when using whole blood for detecting LOY.

However, the sensitivity of all these methods are limited, leaving low frequencies of LOY undetected. These methods require high amounts of DNA input from hundreds of thousands of cells, but LOY is a binary event on the single cell level. The clonal expansion of rare LOY cells could have important clinical implications. Therefore, we aim to combine LOY and cell type detection in a clinically applicable single cell assay. This would benefit both individual patients as well as the health care system and the society at large. The project is funded by an ERC-StG as well as other sources.

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E-P14.06
Implementing Epic, a next generation electronic health record (EHR) at the clinical genetics department, Rigshospitalet Denmark

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Clinical Genetics, Copenhagen, Denmark

When working with implementing a next generation electronic health record as Epic, the establishment of new work flows has been a key strategy. The Epic system is in itself an integration of more than 30 different data systems and devices and the implementation not only requires the hospital staff to adopt new workflows, but also requires patient participation, in order to facilitate their initial enrollment into the patient webportal system. Accordingly, to maximize patient enrollment at the clinical genetics department, we aimed to create new meaningful workflows and foster strong support for the system. We prioritized our efforts on the first patient contact, creating a functioning work flow around the administrators. During the initial enrollment period the administrators communicated that they were hesitant to provide patients with the instructional information pamphlet needed to enroll in EPIC, as they had concerns that it was not up to date and that the necessary functions were not working in Epic. Through implementing weekly meetings with the administrators we were able to consistently correct ongoing problems within the system and make sure that the instructional information given out, corresponded to what the patients met when they accessed the system. Creating this feedback loop ensured a general quality control and maintenance of professional integrity. The potential of using this approach in a managerial or clinical context is apparent.

L. Nicolaisen: None. **B.R. Diness:** None.

E-P14.07

Comparison of nucleic acids extraction to direct amplification of punches processed by the CPA200™ system from NUCLEIC-CARD™ blood and saliva samples

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Introduction: Molecular investigation is important for the detection of clotting defects. The NUCLEIC-CARD™ WHITE (NCW) for blood and COLOR (NCC) for buccal swabs (BS) and the CPA200™ a semi-automated punch-system to process NUCLEIC-CARD™ (NC) (Copan Italia). The study's objective was to compare nucleic acids extraction (NAE) to direct punches amplification (DPA) processed by the CPA200.

Methods: For this study, 10 bloods and BS, were used to standardize the procedure and 35 specimens to validate it. Samples were tested by NAE, and punches, processed by the CPA200 from blood on NCW and BS on NCC, by DPA. Nucleic acid was extracted from blood and BS with the QIAamp® DNA Mini Kit; punches were washed with 200ul sterile water and dried. Extracts and punches were amplified with in-house PCR assays using primers specific for 14 clotting factors (apo e, AGT, FGB, factorV-Y, FACTOR II, FACTOR V LEIDEN, MTHFR677, MTHFR1298, FACTOR V CAMBRIGE, ACE, HPA, PAI, FACTOR XIII, APOB) followed by mini-sequencing reaction and EC on 3500 ABI System.

Results: In the 10 blood and BS all 14 markers were detected using NAE and DPA. In the 35 bloods tested only for FACTOR-II, FACTOR-V-LEIDEN, MTHFR677 and MTHFR1298, were detected by both methods with 100% results correlation.

Conclusions: Data obtained in this study demonstrated that direct punches amplification can detect all 14 clotting markers from NCW punches of blood on NCW and of BS on NCC. The NUCLEIC-CARD™ and CPA200™ in combination with direct punches amplification are facilitating specimens testing workflow for bleeding disorders.

G. Paselli: None.

E-P14.08

biuxx

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In 2016, the Korean government released 46 genes and related 12 phenotypes for direct to consumer (DTC) service. We tested cholesterol markers to make the best prediction model based on our DTC service (GeneStyle™) results. In this study, we describe the testing results from 647 samples. The customers agreed the written confirmed and we collected the buccal swab DNA samples and disease history questionnaire. Among the DTC genes, we examined cholesterol gene markers (*SORT1,HMGCR,ABO,ABCA1, MYL2,LIPG,CETP*) and self-reported hyperlipidemia history with controlling age, sex, smoking, alcohol drinking, family history as the covariates. The single marker association with hyperlipidemia history did not show any association tendencies. Therefore, we combined the seven gene marker genotypes and calculated the genetic risk scores(GRS) based on the number of risk allele and the multiple regression effects size to hyperlipidemia. Interestingly, the combined index of the GRS was significantly associated with the questionnaire cholesterol history (OR 2.492 (95% CI 1.179-5.266), P=0.017). Using this GRS, we estimated the risk prediction accuracy by ROC curve, and the area under cover was 58.8%. Because the most of the DTC markers were studied for the quantitative traits, the predictive power might be weakening. Conclusively, the DTC service genes are well selected by the Korean governments, and the companies should improve the prediction accuracy based on the practical tests.

Keywords: Hyperlipidemia, Genetic polymorphism, DTC

B. Kim: None.

E-P14.10

Vanadium in the blood of SCA2 patients as potential biomarker of the disease

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Introduction: Spinocerebellar ataxia type 2 (SCA2) is a neurological disorder characterized by cerebellar dysfunction. The association between metals and neurodegenerative diseases is under constant investigation, for their involvement in oxidative stress and their potential role as biomarkers of these pathologies. Redox active metals, like vanadium (V), undergo redox-cycling reactions in cells and can produce reactive oxygen species (ROS) in biological systems (Valko et al., 2005).

Materials and Methods: The whole blood of 20 SCA2 patients and of 18 healthy individuals was subjected to V quantification by inductively coupled plasma mass spectrometry (ICP-MS).

Results: Vanadium concentrations were significantly higher ($p< 0.05$) in patients (6.5 ±1.0 micrograms L^{-1}) compared to controls (3.7 ± 0.8 micrograms L^{-1}).

Conclusions: Neurodegenerative processes have been related to metal imbalances, with altered levels of different elements in serum or blood samples. V species and, in particular, the oxido-vanadium cation $VO(^{2+})$ have been previously reported to be involved in the annihilation and formation of reactive oxygen species (Rehder, 2013). The presence of higher levels of V in cells of SCA2 patients may lead to the increase of ROS, and probably to the up regulation of the antioxidant enzymes system to counteract the oxidative stress. In fact, the presence of oxidative stress and a significant increase of superoxide dismutase (SOD1 and SOD2) in SCA2 has been extensively documented (Guevara-Garcia et al., 2012; Cornelius et al., 2017). We suggest that vanadium level in the blood of SCA2 patients is of special interest as potential biomarkers of this disease.

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E-P15 Personalized/Predictive Medicine and Pharmacogenomics

E-P15.03

Antiepileptics-induced serious cutaneous adverse reactions and HLA-B alleles with lymphocyte activation tests in 6 Korean patients

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Introduction: Antiepileptic drugs (AED) have been known to induce serious cutaneous adverse reactions (SCAR) such as Stevens-Johnson syndrome and toxic epidermal necrolysis. Despite of studies for examining the mechanism associated with HLA, the association between AED-induced SCARs and HLA alleles is still unclear. We investigated HLA-B alleles in AED-induced SCARs.

Material and Methods: Six AED-induced SCAR patients were requested for evaluating the causality. Four patients of them were treated with carbamazepine due to pain control. One of the rest was treated with phenytoin, and the other was treated with valproic acid for seizure prevention after cranial haemorrhage operation. All recovered from SCARs after stopping AED treatment and intensive care. HLA-B alleles were performed using PCR-sequence-based typing method and lymphocyte activation test (LAT) was conducted for confirming the culprit drug of the SCARs.

Results: Demographic findings (gender, age), culprit AEDs, and HLA-B alleles of the 6 patients listed in Table 1. LAT results were positive for the culprit drugs except one (F/59). Expression of *HLA-B*51:01* and *HLA-B*15:11* alleles were detected in three (50.0%) and two (33.3%), respectively. Two patients had homozygous *HLA-B*55:01* and *HLA-B*15:11/*55:01* alleles, respectively.

Conclusions: The result suggests that Korean individuals with the *HLA-B*51:01* allele, 8.35% in general population, may be susceptible to AED-induced SCARs. Further investigations are necessary to confirm these findings.

Table 1. HLA-B alleles in patients.

Gender/Age	AEDs	*HLA-B*
F/59	carbamazepine	**15:11/*51:01*
M/60	phenytoin	**51:01/*51:01*
F/48	carbamazepine	**35:01/*51:01*
M/40	valproate	**15:11/*40:06*
F/64	carbamazepine	**46:01/*59:01*
M/67	carbamazepine	**07:02/*58:01*

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E-P15.05

Factors influencing patients' willingness to take predictive breast cancer genetic test: a qualitative study of women in an Asian setting

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Reluctance to take hereditary cancer genetic tests is reported among Asian women. This study aims to understand patient factors influencing the take-up rate of the genetic tests. A total of twenty-four participants were recruited, participants were women with a personal/family history of breast and/or ovarian cancer. shared about the factors that motivated or hindered them from taking the genetic test. Participants were inclined to take the test due to five different reasons - for personal awareness, to create awareness for family, to ascertain their risks of cancer due to a strong family history,

family support and lastly to heed the advice of medical professionals. However, concerns with regards to cost and the perceived lack of ability to cope with the genetic results deterred participants from taking up the test. Additionally, the lack of information about genetic tests was cited by participants as a factor that hindered them from making a decision of whether to take the test.

S. Sun: None.

E-P15.06

Comparison of seven contemporary pharmacogenetic assays with the PharmGKB database

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Genotyping of pharmacogenetic (PGx) genes can provide informed personalized drug therapy and can prevent common adverse drug reactions. Today, there are several tests commercially available to genotype individual Single Nucleotide Polymorphisms (SNPs) in PGx genes. In this study seven modern SNP genotyping assays were compared with the Pharmacogenomics Knowledge Base (PharmGKB) database to determine what proportion of the currently known pharmacogenetic effects is measured by these assays. These seven assays are the "Ion AmpliSeq™ Pharmacogenomics Panel", the "VeraCode® ADME Core Panel", the "iPLEX® PGx Pro Panel", "DMET™ Plus", "PharmcoScan™", "Living DNA", and "23andMe". The PharmGKB database contains 3474 clinical annotations, which describe a variant-drug interaction. 94% of PharmGKB's clinical annotations can be determined with SNP assays. The other part can be determined with haplotypes and copy number variations. Of PharmGKB's clinical annotations, 76%, 68%, and 44% can be determined by PharmacoScan™, Living DNA, and 23andMe respectively. The other described assays, are designed to test only specific subset of PGx SNPs.

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E-P16 Omics/Bioinformatics

E-P16.01

Alu elements as source of microRNA sites in the human genome

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Alu sequences are the most abundant short interspersed repeated elements in the human genome. These sequences are the most abundant mobile elements in the human genome, representing 10.6% of nuclear DNA. The aim of the study was to carry out a full-genomic bioinformational search for microRNA motifs localized in human Alu sequences. Genomic sequences were extracted from the NCBI database using the E-utilities suite of utilities. The micro-RNA sequences were taken from the mirRBase database, release 21. The search was performed using the miRanda and GLAM2Scan software suite. About 80-90% of the miR-619 miR-619, miR-5585, miR-5095, and miR-5096 microRNA binding sites were detected within the Alu elements. The presence of binding sites for certain microRNAs within Alu elements also explains the high conservatism of the distances between sites. The sequences miR-1273g and miR-1285-1,2 were found inside the Alu elements, but lying on the opposite chain. The pre-Mir-1273g sequences were detected within the Alu-elements, which makes the latter a prospective source of the mature miR-1273g. miR-5196 and miR-466 were not found inside the Alu elements, but they are also widely represented in the human genome. The study was supported by the Ministry of education and science of the RF, project № 6.6762.2017.

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E-P16.02

Discovering potential causative mutations in human coding and noncoding genome with the interactive software BasePlayer

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Introduction: Next-generation sequencing (NGS) is routinely applied in life sciences and clinical practice, where interpretation of the resulting massive genomic data has become a critical challenge. The genome-wide mutation analyses enabled by NGS have had a revolutionary impact in revealing the predisposing and driving DNA alterations behind a multitude of disorders. The workflow to identify causative mutations commonly involves phases such as quality filtering, case-control comparison, genome annotation and visual validation, which require multiple

processing steps and usage of various tools and scripts, often available only in Linux environments. To this end, we introduce an interactive and user-friendly multi-platform software, BasePlayer, which allows scientists, regardless of bioinformatics training, to carry out variant analysis in disease genetics settings.

Materials and Methods: The software can be run on Windows, macOS and Linux systems with the Java Runtime Environment 1.7 or newer installed. All annotated reference genomes available at the Ensembl database are supported and can be downloaded and installed through the BasePlayer user interface. Additional annotation files for the human reference genome (GRCh37), such as gnomAD variant control data, ENCODE regulatory regions and predicted transcription factor binding sites are available at the BasePlayer website. The source code is available at https://github.com/rkataine/BasePlayer.

Results: The unpublished version of BasePlayer has already been utilized in at least 18 publications by several disease genetics research groups. The software has now been made publicly available, and developed to be more suitable for a wider audience. BasePlayer can be freely downloaded at https://baseplayer.fi.

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E-P16.03
Protein composition characterization of circulating nucleoprotein complexes

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Background: Cell-free DNA (cfDNA) circulated in bloodstream being packed into membrane coated structures like apoptotic bodies or complexes with biopolymers like histones or DNA-binding plasma proteins [1]. Blood plasma deoxyribonucleoprotein complexes (DNPCs) were isolated by affinity chromatography with antihistone antibodies immobilized on Sepharose 6B; the proteins were separated by 10-20% SDS-PAGE, then the proteins were identified by MALDI-TOF [2,3].

The aims of study: Studying the protein content in DNPCs circulating in the blood plasma of healthy females (HFs)(5 samples) vs primary breast cancer patients (BCPs) (5 samples) by analysis of different protein signatures as well as Gene Ontology (GO) annotation. The analyzed currently MALDI-TOF data were obtained previously [2].

Results: Our results assumed extracellular histone-containing proteins are involved in binding with circulating cfDNA for BCPs over HFs states. HFs: 176 proteins/peptides joined with 195 GO terms were characterized; 45 proteins with no any predicted GO; BCPs: 167 proteins/peptides joined with 168 GO terms were characterized; 39 proteins with no any predicted GO. The most different proteins were characterized by next GOs: GO:0005515 (Ontology:Molecular Function:protein binding):25 proteins for HFs, 33 for BCPs; GO:0006355 (Ontology:Biological Process:regulation of transcription, DNA-templated):8 proteins for HFs, 16 for BCPs; GO:0055085 (Ontology: Biological Process:transmembrane transport):1 proteins for HFs, 5 for BCPs.

References: [1].Bryzgunova OE and Laktionov PP (2014). Generation of blood circulating DNAs: Sources, features of struction and circulation. https://doi.org/10.1134/S1990750814030020

[2].Tamkovich SN, et al (2015). Identification of proteins in blood nucleoprotein complexes. https://doi.org/10.1134/S1068162015060163

[3].Tamkovich SN, et al (2016). Protein Content of Circulating Nucleoprotein Complexes. https://doi.org/10.1007/978-3-319-42044-8_26.

M. Belenikin: None. **S. Tamkovich:** None. **P. Laktionov:** None.

E-P16.04
Epigenetic regulation of NET in postural tachycardia syndrome

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Postural Orthostatic Tachycardia Syndrome (POTS) is characterized by the clinical symptoms of orthostatic intolerance, light-headedness, fatigue and near syncope on upright posture. Abnormal sympathetic nervous system activity suggests dysfunction of the norepinephrine transporter (NET) with evidence the gene responsible is under tight epigenetic control. We show the *Let7i* miRNA is associated with *NET* gene suppression in POTS subjects using genome wide RICh-Seq (RNA of Isolated Chromatin combined with Sequencing). MeCP2 binding is mediated by *Let7i* and subject to pharmacological HDAC inhibition, restoring specific epigenetic modifications associated with *NET* gene expression in POTS subjects. We demonstrate that in POTS subject's pharmacological histone deacetylase

inhibition restores epigenetic control and expression of norepinephrine transporter.

A. El-Osta: None.

E-P16.05

First in-house NGS and bioinformatic analysis of expanded carrier screening in Argentina

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Introduction: Initially, gene-by-gene carrier screening tests were offered to couples with high risk of having a child with a recessive disorder. The advent of next generation sequencing (NGS) has enabled the development of expanded carrier screening tests that analyze multiple genes simultaneously, allowing the consideration of alternative reproductive options and early intervention strategies.

Here we describe the bioinformatic analysis of an expanded carrier screening panel for 688 autosomal and X-linked recessive disorders.

Material and Methods: DNA was extracted from 120 blood samples. NGS of targeted-exons of 483 genes was performed on a Nextseq 550 and sequence data analysis was achieved using an in-house bioinformatic pipeline.

Briefly, data was demultiplexed, converted to Fastq format and low-quality data was removed. Reads were aligned against the reference genome hg19 using Burrows-Wheeler Aligner and Genome Analysis Toolkit was used to call variants. Then, variants were annotated and coverage and functional impact filters were applied using a custom-made script. Filtered variants were classified according to ACMG guidelines.

Results: A mean of 4742 raw variants per sample were found. After filtering, a mean of 355 variants were detected and after variant classification 83 pathogenic/likely pathogenic variants (0.7 per sample) were identified. About one third of the patients were carriers of at least one pathogenic/likely pathogenic variant.

Conclusions: Local development of a bioinformatic pipeline has allowed our laboratory to set up an NGS-based expanded carrier screening test, which could potentially help identify couples at risk and reduce the incidence of severe recessive disorders.

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E-P16.06

Enhancing specificity in genotyping by using a novel two enzyme chemistry

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Here we present an improved SNP genotyping method that combines the hybridization and allele specific extension benefits into a single assay format with universal fluorescent reporter probes. rhAmp^{TM} SNP chemistry relies on the actions of two enzymes, an endoribonuclease (RNase H2) and a mutated DNA polymerase. The rhAmp SNP assay primers contain a single RNA base and are 3' end blocked. In addition, the allele specific primers have a non-target complimentary tail. Rapid de-blocking of the primers by the enzyme RNase H2 only occurs upon hybridization to the perfectly matched target. Once deblocked by RNase H2, the SNP site is then interrogated by a novel DNA polymerase with enhanced allelic discriminating abilities. Upon recognition of the correct base-pairing, extension by the modified polymerase occurs from the perfect match primer. Recent advancements in the technology include: 1) expansion of the design algorithm to design assays against indels, 2) development of a 4-color single tube format and 3) the ability to incorporate degenerate bases in the allele specific primers to compensate for nearby polymorphisms. Those polymorphisms can result in poor assay performance due to: no amplification, weak amplification, or allelic biased detection leading to incorrect genotyping results.

S.D. Rose: A. Employment (full or part-time); Significant; Integrated DNA Technologies.

E-P16.09

Research projects focused Laboratory Information Management System

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Introduction: Multi-project research-focused laboratories like core facilities may face challenge to register, track, integrate and monitor samples and their analyses. These data contain personal information, so secure storage and access has high priority, also due to new European legislation. To face these challenges, we have developed a web-based Laboratory Information Management System (LIMS) optimized for multi-project laboratories.

Results: LIMS is designed as 3-layer web-application, which enhances security and restricts accessibility to potential attackers. Additional computational cluster provides bioinformatic analyses over anonymized genomic data without personal identifiers. Summary analysis reports are immutable to change, but with the possibility of repeated reanalysis in case of upgrades of computational pipelines.

One instance of LIMS can be used to manage several independent projects, with ability to assign user roles for each project separately. This restricts user to access only those projects, samples and analysis, which they are assigned to. Thus, we can collect a large genomic data sets in a single system, which allows to perform wide-scale population studies that are based on aggregation analyses over large cohort of individuals.

LIMS has mechanism to define genealogy relationships between samples, which is utilized in genetics analyses of families' anamneses. Implementation of suggested diseases based on phenotypes terms from HPO database may further assist physicians in decision process.

Conclusions: LIMS is web-based application for facilitate storage and bioinformatic analysis of genomic data with emphasis on security of genomic and personal information. The system is easily extensible over new types and versions of data analysis pipelines.

M. Lichvar: A. Employment (full or part-time); Significant; Geneton Ltd., Bratislava, Slovakia. **R. Hekel:** A. Employment (full or part-time); Significant; Geneton Ltd., Bratislava, Slovakia. **J. Budis:** A. Employment (full or part-time); Significant; Geneton Ltd., Bratislava, Slovakia. **D. Smolak:** A. Employment (full or part-time); Significant; Geneton Ltd., Bratislava, Slovakia. **J. Radvanszky:** A. Employment (full or part-time); Significant; Geneton Ltd., Bratislava, Slovakia. **T. Szemes:** A. Employment (full or part-time); Significant; Geneton Ltd., Bratislava, Slovakia.

E-P16.10
In silico assessment of leukemia: OSM gene and protein analysis

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Introduction: Genetically, in leukemia as cancer with bone marrow origin, oncostatin-M gene plays a crucial role in its manifestation. Leukemia inhibitory factor/oncostatin-M encoded the protein as a secreted cytokine inhibits the proliferation of a number of tumor cells.

Materials and Methods: FGENESH and Promoter Scan were used to the coding sequence and promoter analysis respectively. The encoded protein analyzed by ProtParam and ProtScale and hydrophobicity was assessed by the same ProtScale. Domains were analyzed by CD-search and InterProScan and possible post-translational modifications got evaluated by ScanProsite.

Results: The coding sequence, 759 bp, is located in 45 to 2961 nucleotides. Promoter sequence starts from 824 till 877. Transcription start site is nucleotide number 868 and its distance from ATG is 132bp. The encoded protein is made up 231 acid amines. Arginine and leucine with 13% and selenocysteine and pyro-leucine with 0% were the most and least common ones in protein structure respectively. It is an unstable protein with 30 hours estimated half-life. The protein is hydrophobic in its N-terminal. It includes a domain as 4_helix_cytokine-like_core in C-terminal and 13 post-translational modification sites in 6 different classes. Four phosphorylation sites, three N-myristoylation sites, two amidation sites, a site for leucine-zipper template, two N-glycosylation sites, and a cAMP/cGMP dependent phosphorylation site.

Conclusions: The encoded protein cannot be located in the cytoplasm due to its hydrophobicity and have an extracellular/non-cytoplasmic localization. On the other hand, its instability index shows its time-specific translation which correlates with its function in anti-proliferation cancer cells activity.

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E-P16.11
Network-based classification of neurodevelopmental pathology according to cytogenomic data

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Introduction: Regardless of significant progress in uncovering genomic mechanisms for neurodevelopmental disorders, a large proportion of neurodevelopmental pathology cases remains to be idiopathic. In addition to commonly used analyses of associations between genomic variations and phenotypes, it appears that investigating candidate disease processes by network-based classification according to genomic data represents an intriguing alternative.

Materials and Methods: Genome-wide CNV scan was performed in 545 individuals with neurodevelopmental pathology using array CGH and SNP-array techniques. Network-based classification of probable functional consequences of genomic rearrangements was done according to a previously described protocol (Yurov et al., 2017) based on the fusion of genomic, epigenomic, proteomic/interactomic and metabolomic data.

Results: We found that the variomes (set of all CNVs in an individual genome) of individuals with neurodevelopmental pathology are enriched in DNA replication, DNA damage/repair, nucleotide excision/mismatch repair, ATM-pathway (DNA damage-ATM-p53-apoptosis pathway) as well as p53-, MAPK-, ErbB-, PI3KAkt-signaling pathways. Additionally, we found enrichment for pathway clusters or combinations (e.g. MAPK-signaling with axonal guidance; DNA damage-ATM-p53-apoptosis with phagosome and gap junction; p53-signaling with RNA transport and pathways in cancer etc.). There were 12 distinct pathway/process clusters and 14 distinct pathway/process combinations which were further used for classification of neurodevelopmental pathology.

Conclusions: Network-based classification of neurodevelopmental pathology according to cytogenomic data appears to be an efficient approach to determine candidate processes for neurodevelopmental diseases. Furthermore, our data indicate, for the first time, that alterations to process clusters and combinations of altered processes could be mechanisms for abnormal brain functioning. Supported by RSF (14-15-00411).

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E-P16.13
Annotator: a novel custom tool for genomic variants annotation and classification

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Introduction: Genome sequencing produces large amounts of data that enable the discovery of genomic variants. Using appropriate bioinformatics tools and public databases which aggregate clinical information, it is possible to understand the functional impact of such variants on human phenotypic traits. Several bioinformatics tools already exist, however with limitations such as, the license costs and the limited choice of public databases consulted. Therefore, our aim was to implement an in-house tool that aggregates information collected from selected public databases, allowing the custom annotation of genomic variants and their classification in terms of pathogenicity.

Materials and Methods: We implemented a tool, Annotator, which annotates and classifies genomic variants using data from 5 renowned public databases (Uniprot, OMIM, ClinVar, dbSNP, Pubmed). Annotator can also integrate keywords, defined according to sample characteristics, for advanced data/text mining, in order to further select collected data to best suit the clinical features of the sample under analysis.

Results: Annotator was used to analyze and classify a set of 151 genomic variants, detected in 52 probands with Familial Intestinal Gastric Cancer. These genomic variants had already been analyzed/classified using a commercial software. The comparison of the results obtained with both analyses showed that Annotator was able to collect further information for 7/42 somatic variants and 4/24 germline variants, which were classified as of unknown significance for the commercial software.

Conclusion: Annotator is a valid tool for an accurate annotation and efficient classification of genomic variants derived from sequencing experiments.

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E-P17 Epigenetics and Gene Regulation

E-P17.01

Fresh garlic extract might trigger apoptosis trough down regulation of miR-146a in A549 lung carcinoma cells

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Obective: Garlic (Allium sativum L.) has been used as herbal drug since ancient ages for pulmonary and respiratory complaints. Its extract Ally methyl sulfide (AMS) gives characteristic odor on breath when taken orally and known to have anti-proliferative and apoptotic effects on lung carcinoma cell lines (A549). However, mechanism still needs to be elucidated. Thus, we aimed to explore the apoptosis regulating miRNA expressions for apoptotic effects of garlic extract on A549 cells.

Methods: A549 cells were incubated with fresh garlic extract for 24 h. Total RNAs were extracted from cell cultures with cell count of 1x10E5 in 6-well plates. 10 miRNAs regulation apoptosis were selected (miR-21_2, miR-31_1, miR-39_1, miR-133a_2, miR-143_1, miR-146a_1, miR-155_2, miR-183_2, miR-210_1 and miR-222_2) and expressions were analyzed by real time PCR (RotorGene Q, Qiagen).

Results: Fresh garlic extract treated A549 cells represented decreased expressions of miR-146a_1 (3,47 Fold decrease) as the apoptosis increased. However, proapoptotic miR143 expressions were also downregulated.

Discussion/Conclusion: Our study was the first to link the apoptosis of lung carcinoma cell lines with miRNA expressions in A549 cell line, up to the literature. According to our results apoptosis might be triggered by upregulation of FasL due to down regulation of miR146a in garlic extract treated A549 lung cancer cells. Down regulation of proapoptotic miR143 might be a compensation mechanism and need to be investigated with further studies. REFERENCE: Gruhlke MC, et al. Antioxidants (Basel). 2016 Dec 26;6(1)

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E-P17.02

Propolis might induce apoptosis through downregulation of anti-apoptotic miRNAs on A549 human lung carcinoma cells

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Subject: Propolis is honey bee-derived apicultural product that have been applied for human health for centuries in traditional medicine besides food diets and supplementary nutrition. It has also attracted researchers' attention due to its apoptotic and anti-carcinogenic properties. Here, we aimed to investigate the mechanism of apoptotic effects of propolis by means of apoptosis regulating miRNAs expressions in human lung carcinoma cell lines (A549 cells).

Methods: Propolis extracts (beeMYHoney) were prepared by ethanol (250 µg/mL). A549 cells were incubated with propolis extracts for 24 h with different concentrations in cell cultures of 6-well plates using 10x10E5 cells/well. Total RNAs were extracted and 10 miRNAs expressions were analyzed by real time PCR (RotorGene Q, Qiagen).

Results: The optimum apoptotic affects were achieved at concentrations of 0,625 µg/mL. Propolis extract treated A549 cells showed gradually increased expressions of miR-133a_2 and miR-143_1, and gradually decreased expressions of miR-21_2, miR -146a_1, miR-155_2 and miR-183_2 by increased dosages of 0,125 µg/mL to 0,625 µg/mL compared to non-treated tumor cells .

Conclusion: In our study, we for the first time represented the downregulation of apoptosis regulating miRNAs in lung cancer cell lines (A549) after treatment of propolis extract, up to the literature. Hence, they may be a role for propolis to induce apoptosis (as its anti-tumorigenic affect) through epigenetic changes on Fas and FasL with/without PTEN on the etrinsic pathway of programmed cell death.

Reference:

Preventive and protective effects of Turkish propolis…. Acta Biol Hung. 2011. 62(4):388-96.

Degradation of miR-21 induces apoptosis…Cancer Gene Ther. 2015 Nov;22(11):530-5.

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E-P18 Genetic epidemiology/Population genetics/Statistical methodology and evolutionary genetics

E-P18.01

The frequency of change of the nucleotide sequence in the gene *TYR* at albinism in Russia

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Introduction: Albinism is a genetically heterogeneous hereditary disease characterized by a reduced amount of melanin pigment in different structures of the body. Currently, the albinism is described in either an isolated form affecting the only eye or eye and skin (11 clinical and genetic variants), or syndromic pathology. According to the literature, about 70% of cases account for the oculocutaneous form. In 5% of cases, a reduced amount of pigment is a symptom of a syndromic pathology.

Aim: To investigate the frequency and spectrum of the mutations in the *TYR* gene in Russian patients with albinism.

Materials and Methods: The laboratory of genetic epidemiology, "RCMG," for the first time in Russia conducted a clinical and genetic study of patients with albinism. The cohort size was 33 Russian patients. We used clinical and molecular-genetic methods to confirm the diagnosis.

Results: The molecular genetic study of DNA of the patients revealed changes in the *TYR*, which is responsible for oculocutaneous albinism type 1, are the prevalent cause (63%). Frequent mutations were c.650G>A (frequency 0.42), c.1037-3C>G (0.15), c.140G>A (0.07). Changes in gene *OCA2* were observed in 9% cases (oculocutaneous albinism type 2). In other 9 % of cases, isolated ocular albinism associated with changes in *GPR143* was confirmed. In the latter two types, no frequent mutations have yet identified.

Conclusions: Russian patients with albinism have the high frequency of mutations in the *TYR* gene (63 %) associated with oculocutaneous albinism type 1. The work was partially supported by the RFBR grant 17-04-00288.

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E-P18.03

Investigation of *MCT1* rs1049434, *COL1A1* rs1800012 and *COL3A1* rs1800255 variants related to susceptibility to injuries in professional football players

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Introduction: Some variations in monocarboxylate transporter (*MCT1*) and collagen-encoding (*COL1A1*, *COL3A1*) genes have been associated with specific athletic performance and susceptibility to injuries (e.g. musculoskeletal soft tissue injuries). The aim of this case-control association study was to investigate prevalence of risk alleles of *MCT1* rs1049434, *COL1A1* rs1800012, *COL3A1* rs1800255 in Lithuanian professional football players.

Methods: A total of 150 footballers (Caucasian male) and 150 controls (sedentary, healthy male) were genotyped using *TaqMan* RT-PCR assay.

Results: The genotype distribution were in HWE for all groups (P>0.05). There were no differences in genotype/alleles frequency for *COL1A1* rs1800012 and *COL3A1* rs1800255 between the footballers and control groups. The odds ratio (OR) of athlete harboring *COL3A1* AA genotypes (the risk allele A) compared to control was 0.29 (95%CI: 0.08-0.89, $p = 0.04$). Significant *MCT1* genotype distribution were determined between the footballers and controls (TT/TA/AA: 45.3/44.7/10% vs 34.0/47.3/18.7%; $p = 0.04$). The proportion of *MCT1* risk allele A, observed in controls (42.3%) was larger than in all footballers (32.3%, $p = 0.07$), specifically, than in midfielders (26.7%; $p = 0.04$). The OR of *MCT1* TT genotype and being a football player was 1.61 (95%CI 1.012-2.574, $p = 0.045$), while the OR of *MCT1* AA genotype was 0.48 (95%CI 0.242-0.937, $p = 0.035$).

Conclusions: *MCT1* rs1049434 (T allele) associated with football performance. Carrying the *MCT1* TT genotype may be protective against sport-related injury. The *MCT1* AA and *COL3A1* AA genotypes may influence increased risk of injury. Replication studies are needed to support our data and to fully understand the relationship between predisposition to injuries in football.

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E-P18.04

Identification of the genetic factors underlying comorbidity between bronchial asthma and hypertension

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The phenomenon of comorbidity between diseases in an individual patient is a global problem of contemporary health care. The comorbidity between two phenotypes can be explained by shared genetic variants predisposing to both diseases. One of the features of the clinical course of bronchial asthma (BA) is its significant comorbidity with cardiovascular pathology. According to epidemiological data, BA has a decisive influence on the subsequent risk of cardiovascular disease. Therefore, hypertension and BA can be considered as an example of common comorbid diseases. From the point of view of genetics, these two diseases are well studied and many genes for BA and hypertension were identified. We assume that their comorbidity is based on shared pathophysiological changes for both BA and hypertension associated with genes involved in the regulation of immune system, smooth muscle tone and vascular remodeling. We set out to elaborate this hypothesis and identify genes of this comorbidity. We prioritized genes using several criteria based on gene network analysis describing mechanisms of BA and hypertension comorbidity. The top list of candidate genes that can be involved in convergent pathophysiological mechanisms leading to comorbidity of BA and hypertension include *TLR4, CAT, IL10, CST3, ICAM1, IRF6, AKT1, NFKB1, PNP, SELL, CCL5, IL2RB, IDS, FOS, NT5C2*, and *BHLHE40*. We also identified 96 eQTL SNPs for these genes and study them to validate our hypothesis in cohorts of patients with BA, hypertension and individuals affected by both the diseases. This work was funded by VolkswagenStiftung program (grant №90335).

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E-P18.05

The impact of prenatal diagnosis on congenital malformations prevalence in Russia

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Aim of the study. The impact of prenatal diagnosis on prevalence of congenital malformations (CM).

Materials and Methods: The materials of the study were cases of CM among live births, stillbirths and induced abortions registered in the regions of the Russia for the 2010 to 2014. The total number of births was 3030104. The analysis includes: anencephaly - 138 cases, encephalocele - 106, spina bifida - 878, hydrocephalus - 1095, omphalocele - 288, gastroschisis - 345, renal agenesis - 78, transposition of the great vessels - 523, hypoplastic left heart - 324, diaphragmatic hernia - 564, bladder extrophy - 59, cleft lip - 2157, cleft palate - 1404, limb reduction defects - 1036, esophageal atresia - 721, anorectal atresia - 708, hypospadias - 4141, epispadias - 30 and Down syndrome - 2842.

Results: The prevalence with and without elective pregnancy terminations was detected for each defect. The high level of pregnancy termination occurs for anencephaly, encephalocele, bilateral renal agenesis. The rate of pregnancy termination for Down syndrome is 34%. As a result of the including of induced abortion the rate of defects varies greatly: for anencephaly (in 7.48 times), encephalocele (3.18 times), renal agenesis (2.53 times), omphalocele (2.39 times). The Down syndrome rate in newborns decreased by 1.5 times among livebirths.

Conclusion: The accounting of CM among newborns and elective terminations allows to determine the true prevalence of congenital malformations and to assess the impact of prenatal diagnosis on the level of the CM among newborns.

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E-P18.07

The relationship between genomic damage and eating behavior in healthy middle-aged Koreans

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Introduction: Cytokinesis-block micronucleus cytome (CBMN-Cyt) is a quantitative index of DNA damage. The purpose of this study is to know the correlation between DNA damage and eating habits in healthy middle-aged Koreans.

Materials and Methods: DNA damage was evaluated by the frequency of micronuclei (MNi), nucleoplasmic bridges (NPBs), nuclear buds (NBUDs) with CBMN-Cyt assay in totally 300 healthy males and females aged 30-59 years. There were no smokers nor problem drinkers. The relationship between participants' eating behaviors and CBMN-Cyt assay parameters were analyzed by linear regression model.

Results: In univariate analysis, in men, MNi frequencies were higher with regular intake of vegetable, and lower with regular intake of high animal fat and high sugar desserts

(P<0.05). In women, NPBs frequencies were higher with regular intake of vegetables, and NBUDs frequencies were higher with regular intake of protein and diverse kinds of food (P<0.05). After adjusting age, job and exercise behaviors, although MNi and NBUDs frequencies were not related of each eating behaviors, NPBs frequencies were higher with regular intake of fried food or stir-fry (P=0.020) in men. In women, while MNi and NPBs frequencies were not related of each eating behaviors, NBUDs frequencies were higher with regular intake of protein (P=0.034).

Conclusion: Generally, in 30-59-year-old healthy Koreans with genomic healthy lifestyles, there were no significant relationships between DNA damage parameters and eating habits, but the regular intake of fried food or stir-fry in men and regular intake of protein in women were associated with the increase of DNA damage.

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E-P18.09

Allele frequencies of *PAH* gene mutations in patients with hyperphenylalaninemia in Republic of North Ossetia Alania (Russia)

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Introduction: Hyperphenylalaninemia (HPA) is an inborn metabolic condition, caused by mutations in the some genes (PAH, PTS, QDPR, GCH1, PCBD1, SPR). That results in decreased metabolism of phenylalanine (FA) and can lead to the severe intellectual deficiency.

Aims: To study the frequency of HPA in the Republic of North Ossetia-Alania (NOA) according to the results of newborn screening. To assess the frequency and the spectrum of the PAH mutations in unrelated patients with HPA.

Materials and Methods: The number of newborns in the NOA screened for the period 2007-2016 was 101005. 28 patients with HPA were identified. The frequency and the spectrum of mutations in the PAH gene was studied in 14 unrelated individuals of HPA.

Results: The frequency of PKU according to the results of newborn screening was 1:3607 newborns. A DNA study conducted in 14 patients with HPA revealed 11 mutations. Two mutations were detected with high frequencies: P281L (allele frequency 0.43), P211T (0.21). Mutation R261Q (0.07) was found in two patients in the compound heterozygous state with mutation P281L. The remaining 8 mutations (V230I, A403V, R408W, F331S, E390G, A300S, R261Q, M1R, I306V, R261Q) met once (allele frequency 0.04).

Conclusions: Newborn screening revealed a high incidence of HPA (1:3607 newborns) in NOA. The population of NOA is characterized by a specific spectrum of the PAH mutations; frequent ones are P281L and P211T. This work was partially funded by RFBR grant 18-15-00090.

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E-P18.10

Distribution of Factor V Leiden, Prothrombin G20210A and MTHFR C677T mutations in ethnic Georgian, Armenian and Azerbaijani Patients living in Georgia with thrombosis or pregnancy complications

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Introduction: Thrombophilia gene mutations are most common genetic factors of thromboembolism and pregnancy complications. The prevalence of these mutations differs significantly in populations and ethnic groups.

Aim: Due to the fact that ethnic Georgians (86,6%), Azerbaijanis (6,3%) and Armenians (4,5%) are living together in Georgia and lack of data on the prevalence of thrombophilia gene mutations among these ethnic groups, the aim of our study was to detect and compare distribution of thrombophilia gene mutations in Georgian, Armenian and Azerbaijani Patients with thrombosis or pregnancy complications.

Materials and Methods: 548 Georgians, 56 Armenians and 122 Azerbaijanis with venous thromboembolism or pregnancy complications and also 200 unaffected Georgians were genotyped by PCR analyses.

Results: Our eight years studies confirm that distribution of studied mutations in Georgians resembles upper data of Caucasians and has impact on the development of thrombosis and pregnancy complications. Prevalence of studied mutations is different in these ethnic groups, with highest rate in Armenians. Results are presented in table.

Distribution of mutations in ethnic groups of Georgia

Ethnic Groups of Georgia	FVL-1691A	Pr-20210A	MTHFR-677T (homo)	MTHFR-677T (hetero)
Georgian (patients) (548)	11.5%	7.12%	9.85%	37.4%
Georgian (control) (200)	1%	3%	2%	35%
Armenian (patients) (56)	28.57%	17.86%	14.29%	42.86%
Azerbaijanis (patients) (122)	8.2%	3.28%	6.56%	39.34%

Conclusions: It is important to investigate whether this highest rate of mutations in Armenians is due to the phenomenon of genetic isolates or the rate is the same as in Armenian population. Following our results, large-scale research of thrombophilia gene mutations in Armenian and Azerbaijani population is reasonable, because prophylaxis and timely started treatment is very important for prevention of thromboembolism and pregnancy complications.

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E-P18.14

Insight into genetic and social aspects of modern communities of deaf people in Siberia for forecasting the prevalence of hereditary deafness

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Genetic deafness is heterogeneous disability with different inheritance patterns and the most common form is recessive deafness caused by mutations in gene *GJB2*. High frequency of *GJB2*-caused deafness in some populations is suggested by combined effects of assortative mating tradition among deaf people based on linguistic homogamy (sigh language) and relaxed selection against deafness (Nance et al., 2000). We previously revealed *GJB2*-caused deafness proportion in some indigenous Siberian populations: 15.1% in Tuvinians (Republic Tuva), 17.5% in Altaians (Republic Altai), 53.0% in Yakuts (Republic Sakha/Yakutia) while sufficient number of familial deafness cases remained undiagnosed. This study presents the results of comparative analysis of the survey data (marriage patterns, fertility, communication mode, social interactions) of deaf individuals from these Siberian regions. Assortative marriage rates were 64.5% (Tuva), 72.9% (Altai), with significant differences ($p<0.05$) between urban and rural residents - 70.9% vs 57.7% (Tuva), 91.9% vs 58.3% (Altai), and 77.1% (Yakutia, urban residents). Fertility (mean children number) of deaf individuals is slightly reduced compared to their hearing siblings: 2.22±0.06 vs 2.40±0.05 (Tuva), 1.76 ±0.10 vs 2.24±0.09 (Yakutia). Majority of respondents indicate sign language as main communication mode and prefer contacts with deaf people whereas they feel embarrassed by misunderstandings with hearing people. We developed an agent-based computer model to study the trends of hereditary deafness prevalence in population taking into account obtained genetic and socio-demographic data. Model is designed for single-locus control of deafness with possibility of extending for two or more loci. Work is supported by ICG project #0324-2018-0016 and RFBR (#15-04-04860_a, #17-29-06016_ofi_m).

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E-P18.15

Blood glucose, BMI and telomere length in a cohort of Colombian schoolchildren

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Introduction: A few studies have reported that mean telomere length (TL) is associated with obesity and blood glucose in children of European or Arab origin. The present study was designed to assess whether BMI or blood glucose is associated with TL in school-age children of South American origin.

Materials and Methods: Relative telomere length ratios were determined in 375 subjects (age 9-14 years). Subjects were recruited within the ACFIES (Association between Cardiorespiratory Fitness, Muscular Strength and Body Composition with Metabolic Risk Factors in Colombian Children) study. Phenotypes were determined using standard tests and an extensive questionnaire. Statistical analysis was carried out using SPSSv25 (IBM Inc., USA).

Results: Partial correlation analysis, controlling for the effects of age, gender, PCR plate and BMI demonstrates a robust negative correlation between TL and blood glucose ($r = -0.14$, $p = 0.008$). Using a univariate linear model analytical approach, blood glucose is also significantly associated with telomere length ($p = 0.011$) with the same variables in the model. No significant association with BMI was detected.

Conclusions: TL is a potential biomarker: blood glucose is negatively associated with TL in this cohort of Colombian schoolchildren and this effect does not appear to be related to obesity. The lack of association with BMI may be due to the difference in ethnicity or because previous studies have had higher than normal proportions of children with early-onset obesity. Further work to investigate this relationship in larger cohorts may yield insights into the relationship of telomere attrition with pathological processes in pre-clinical diabetes.

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E-P18.16
The study of *CFTR* mutations in the population of the North Ossetia Alania Republic, Russian Federation

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Introduction: Cystic fibrosis (CF; OMIM #219700) is a common autosomal recessive disease caused by mutations in the CFTR gene. The prevalence of *CFTR* mutations differs among various ethnic, demographic, and racial groups. Peculiar distribution of CFTR mutations in Ossetians was studied. The Republic of North Ossetia – Alania, a part of the North Caucasus Federal region of RF, is located on the Northern slope of the Greater Caucasus. The population is about 700,000 people. Ossetians make 64,5% of the population of North Ossetia.

Materials and Methods: DNA of 6 Ossetian CF patients from the Republic of North Ossetia – Alania was analyzed for 33 common CFTR mutations and sequencing. DNA of 108 healthy Ossetians without CF family history was tested for 13 CFTR mutations (CFTRdele2,3(21kb), F508del, I507del, 1677delTA, 2143delT, 2183AA>G, 2184insA, 394delTT, 3821delT, L138ins, W1282X, E92K).

Results: Three CF patients were relatives: two first siblings and one second sibling. They had the same genotype, F508del/W1282X. The genotypes of three other unrelated CF patients were W1282X/W1282X, W1282X/2184insA, 2184del4/1248+1G>A. One heterozygous carrier of W1282X mutation was detected in 108 healthy Ossetians.

Conclusion: The most common CF mutation in Ossetian patients was W1282X (4 in 9 CF chromosomes). Earlier W1282X mutation was also revealed in some other Caucasus populations (Karachay, Nogai). 1677delTA mutation, common in autochthonous Caucasus ethnic groups, such as Chechens, Ingush, Georgians, was not identified either in Ossetian CF patients or in healthy persons. 2184del4 and 1248+1G>A mutations are novel for Russian Federation.

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E-P19 Genetic counselling/Education/public services

E-P19.01
Prenatal findings of 28 infants with Down syndrome: a two-year retrospective evaluation

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Introduction: Down syndrome (DS) is the most common chromosomal disorder with the birth incidence 1:700-1000. The high risk of DS is evaluated by prenatal screening programs during pregnancy. The aim of this study is to summarize the prenatal findings of 28 infants with Down syndrome investigated postnatally.

Materials and Methods: The study includes a retrospective data analysis of intrauterin periods of 28 babies with DS. Conventional karyotypes from peripheral blood samplings were performed to confirm DS diagnosis.

Results: The prenatal karyotype indications of the families are shown in Table 1. Only three DS cases were prenatally diagnosed.

Table 1. Indications for prenatal Down syndrome screening in the families

Maternal age	Indications of prenatal karyotyping				Indication Ø
	Maternal age alone	First and/or second maternal serum screening	Ultrasound anomalies	Combined*	
< 35 years	0	2	5	4	5
≥ 35 years	3	4	4	1	0

*Positive serum screening with abnormal ultrasound findings

Among the 28 families, 23 (82%) were evaluated in the high-risk group, 5 (18%) were in the low-risk group. However, only three families have had amniocentesis. Major congenital malformations were detected postnataly in 22 DS infants (79%).

Conclusion: Prenatal and postnatal genetic counseling is extremely important in Down syndrome. Prenataly detection of severe malformations is one of the most important factor affecting family decisions about whether or not to continue the pregnancy.

H. Ilgin-Ruhi: None.

E-P19.02

Pre-eclampsia: Awareness of the life threating condition Pre-eclampsia: awareness of the life threating conditionPre-eclampsia: Awareness of the life threating condition

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Introduction: Pre-eclampsia is categorized as a hypertensive disorder occurring during pregnancy. Mild forms of preeclampsia generally do not possess any symptoms, whereas the severe forms may cause headaches, renal insufficiency and even fetal and maternal death. The incidence of pre-eclampsia varies between 2% to 10% of pregnancies globally. However, WHO reported that this rate is reported to be almost seven times more in the developing countries. Therefore, this study aims to investigate the knowledge, screening methods and the incidence of pre-eclampsia in North Cyprus.

Materials and Methods: Questionnaire based survey were conducted to women at least 18 years of age. The maternal variables including age, possible gestational age and previous pregnancy associated complications were collected from all the volunteers. The social-economic and educational status of the women were taken into consideration for this analysis. The knowledge and awareness of pre-eclampsia associated risks and complications were evaluated.

Results: The overall awareness of pre-eclampsia was 53% and 39% had experienced pre-eclampsia themselves. Due to the lack of knowledge on the complications, women with pre-eclampsia specified that they experienced substantial panic and stress upon diagnosed. The awareness was associated with the level of education. However, generally the women were not aware of the maternal/fetal mortality risk or any possibilities with the genetic predispositions.

Conclusion: Lack of awareness translates to worse health outcomes, including fetal and maternal death or pre-term births. Therefore, proper counselling should be provided to the community about the incidence and the indication of this threatening condition.

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E-P19.03

Clinical Genetics and rare diseases in University Hospital Brno, Czech Republic (Genetic counselling - Diagnostics - Care - Research - Education)

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In accordance with the, Council Recommendation on an action in the field of rare diseases" the Czech Government approved the 2nd National Strategy for rare diseases. The Department of Medical Genetics University Hospital Brno cooperates with the National Coordination Centre for Rare Diseases (Prague-Motol) and with centres for rare diseases in University Hospital Brno (part of respective European Reference Networks), e.g. mainly in the area of geno-dermatoses and epidermolysis bullosa, cystic fibrosis, neuromuscular diseases, rare cardiac arrhythmias and malignant hyperthermia. Clinical geneticists and molecular biologists are involved in genetic counselling, provide postnatal and prenatal genetic diagnostics and preventive testing in families with various rare diseases. In cooperation with the Czech Society of Medical Genetics and Genomics (www.slg.cz) our department organises an annual conference for professionals in the field of medical genetics on the topic of rare diseases. Rare diseases are also the theme of lectures for students of the Faculty of Medicine and Faculty of Science, Masaryk University (MU) in Brno. In cooperation with patient organisations, as DEBRA CR, Czech CF Association, Parent-Project and others, we prepare lectures and informational materials for patients with rare diseases and their families. Together with the Czech Association of Rare Diseases (www.vzacna-onemocneni.cz), we organize lectures for students of Masaryk University in 2017 in which are patients with rare diseases directly involved and interact with students. An integral part of our work are also lectures for the public and an annual meeting in Brno organized on the occasion of the annual Rare Diseases Day.

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E-P19.04
The effect of neuroeducational methods on leucocyte telomere shortening dynamics

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Introduction: Recent data points the importance of psychological factors for health state including genetic level. The aim of this study is to investigate the effect of neuroeducational methods on telomere length shortening.

Materials and Methods: This study summarizes the findings on telomere length changes from a controlled group study of neuroeducational methods over a 6 month period. The study was conducted on 20 relatively healthy subjects aged 20-59 years old. 10 persons had regular neuroeducational sesions (stress reduction, mindfulness, art therapy with professional specialists). Control group consisted of 10 individuals, matched by demografic and health history criteria. HT-Q-FISH was used to measure the median telomere length (LifeLenght, Spain)

Results: Subjects that have passed neuroeducational sessions decreased telomere length over the 6 months period non significantly (100±27bp; $p = 0.63$), whereas subjects in the control group significantly lost telomeres (420±80bp; $p = 0.02$).

Conclusions: The findings of this pilot study suggest that neuroeducational methods can contribute for the slower shortening of telomeres.

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