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Electronic Posters

E-P01
Reproductive Genetics/Prenatal Genetics

E-P01.01
A Novel Missense Mutation in the CPS1 Gene Causes arbamoyl phosphate synthetase 1 deficiency identified by next generation sequencing

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A Novel Missense Mutation in the CPS1 Gene Causes Carbamoyl phosphate synthetase 1 deficiency identified by next generation sequencing

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Introduction: Carbamoyl phosphate synthetase 1 (CPS1) is a liver-specific enzyme with the lowest enzymatic rate, which determines the overall rate of the other reactions in the pathway that converts ammonia to carbamoyl phosphate in the first step of the urea cycle. Carbamoyl-phosphate synthetase 1 deficiency which usually presents as lethal hyperammonemia, is a rare autosomal recessive hereditary disease. Here we report a case of a two-day-old female neonate with lethal hyperammonemia. The newborn infant presented with hyperammonemia.

Materials and Methods: We performed SNParray and after that we performed a WES based on NGS. Sanger sequencing to confirm the mutation was in the patient. The mutation was checked in her parent and other family members too.

Results: we found a novel missense c. 2758G>C mutation in exon 23 of CPS1 at amino acid position 920 (p. Asp920His). We used the Sanger sequencing to confirm the mutation was in the patient. The mutation was checked in her parent and other family members too.

Conclusions: We applied WES successfully to diagnose the patient with CPS1D in a clinical setting. This result supports the clinical applicability of WES for cost-effective molecular diagnosis of UCDs in prenatal diagnosis to future procedures of disease-free embryo selection.

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E-P01.05

Developing comprehensive preconception carrier screening for 21-hydroxylase-deficient congenital adrenal hyperplasia in Thai population

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Introduction: 21-hydroxylase-deficient Congenital Adrenal Hyperplasia (21-OHD CAH) is an autosomal recessive disorder causing by mutations in *CYP21A2*. This condition has diverse severity, ranging from classical severe neonatal life-threatening to mild non-classical form. The most severe classical form can elicit salt-wasting crises in the newborn. Additionally, classical 21-OHD CAH is able to induce simple virilization resulting in ambiguous genitalia in females and precocious puberty in males. Identifying couple-at-risk at preconception stage will have a benefit on prenatal fetal monitoring and treatment.

Materials and Methods: We developed a rapid molecular method to detect 9 common *CYP21A2* mutations which have been reported as common etiologies for both classical and non-classical forms using Amplification-Refractory Mutation System containing P30L, Int2G, G110del8nt, I172N, V281L, Q318X, R356W, P453S and exon 6 cluster variants (I236N, V237E and M239K). Null variant or large gene deletion testing was performed by real-time PCR and Restriction Fragment Length Polymorphism. These screening tools were then tested in 50 enrolled subjects at preconception genetics clinic.

Results: The developed techniques revealed the consistent results to standard methods and controls. Two of 50 subjects (1/25) were identified as 21-OHD CAH carrier. Of them, one was interpreted as a carrier for large-scale gene deletion, whereas another case carried 2 variants locate within the adjacent exons, Q318X and R356W which was likely *in cis*.

Conclusion: Carrier screening for 21-OHD CAH using mentioned techniques were reliable and rapid. The cost was also competitive to be proposed as a national screening.

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E-P01.06

Expanded carrier screening in Belgium: intentions and attitudes of potential users visiting their gynaecologist

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In Belgium, the Superior Health Council (SHC) recommended that expanded carrier screening (ECS) should be available to all couples considering a pregnancy. Little is known about the attitudes of potential users for such an offer within the Belgian population. The purpose of this study is to assess attitudes and interest of potential users regarding preconceptional ECS and to explore determinants related to the intention of accepting preconceptional ECS. Women (aged 18–40 years) consulting their gynaecologist will be invited to complete a self-administered questionnaire that was developed based on the Theory of Planned Behavior and existing questionnaires previously used in other studies assessing attitudes regarding ECS. Participants will be informed about their population risk of being a carrier couple, the risk of conceiving a child with a genetic condition for carrier couples and reproductive options available for carrier couples prior to filling in the questionnaire. Descriptive statistics will be used to describe characteristics of participants. Independent sample t-test (continuous data) and Chi-Square test (categorical data) will be used to compare participants with and without intention to have preconceptional ECS. Logistic regression analyses will be performed to determine which independent variables have a significant predictive effect on the intention to have preconceptional ECS. The results of this study will contribute to a better understanding of the attitudes of potential users and support the implementation of preconceptional ECS in the Belgian healthcare system.

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E-P01.07

Prenatal diagnosis of isolated Congenital Heart Defects: results of a prospective study genome-wide high resolution array-CGH versus karyotyping and 22q11 FISH

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Objectives: We evaluate the relevance of array-based comparative genomic hybridization (aCGH) as a diagnostic prenatal tool in isolated congenital heart defect (CHD) by comparing chromosomic imbalance rate (Copy Number Variations: CNVs) identified by aCGH versus karyotyping/ 22q11 fluorescent in-situ hybridization (FISH).

Methods: In this prospective study, 78 pregnant women whose fetus had an isolated CHD were recruited. Each patient underwent fetal specialized ultrasound scans, genetic counselling and amniocentesis. On the same sample, karyotyping, 22q11 FISH analysis and a-CGH were performed.

Results: Out of the 78 fetuses, 15 CNV (19.23%) were detected by aCGH and only 5 (6.41%) were identified by karyotyping / 22q11 FISH ($p = 0.0001$). CNVs were interpreted as likely benign in 7/78 (8.97%), as Variants of unknown significance (VOUS) in 2/78 (2.56%), and as pathogenic in 6/78 (7.69%). Finally, only 5 fetus with 22q11 deletion were found out by aCGH as well as FISH.

Conclusion: These results illustrate the complexity of identifying a new pathogenic CNV when inclusion criteria are strict. Even if the study couldn't demonstrate the usefulness of aCGH, this tool emerges as an appealing alternative to karyotyping in prenatal isolated CHD.

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E-P01.09

Mosaic sex chromosomes aneuploidies indirect detection in pregnant women by Non-Invasive Prenatal Testing (NIPT)

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Introduction: In cases with a high-risk result of X chromosome aneuploidy obtained by Non-Invasive Prenatal Testing, if the pregnant woman's karyotype is unknown, is interesting to determine, in the maternal blood sample, if the NIPT result is a consequence of an X-chromosome aneuploidy present in the pregnant woman.

Material and Methods: Fluorescent in situ hybridization (FISH) technique was performed in the buffy coat, exclusively containing maternal platelets and leukocytes, of three pregnant women blood sample (Cell -Free DNA BTC® tube); two of them with male fetal sex and X-chromosome high risk result in the NIPT (45,Y), and the other, with X-chromosome trisomy high risk (47,XXX).

Results: The FISH study performed in the maternal blood cells of the two samples with 45,Y NIPT result, showed presence of 83.6% of X-monosomic cells (45,X) in one and 75% in the other. FISH analysis performed in the sample with X-trisomy high risk showed 90% of the maternal blood cells with 47,XXX.

Conclusion: Non Invasive Prenatal Testing in maternal blood sample detects, in some cases, the presence of an aneuploidy mosaicism in the pregnant woman, that compromise the result obtained by NIPT.

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E-P01.10

The benefits of using a targeted sequencing in non-invasive prenatal testing—two years' experience of constellation laboratory in Czech Republic

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Introduction: Nowadays we are witnessing the rapid penetration of non-invasive prenatal diagnostics into routine practice. In the past years, many types of cell free DNA (cfDNA) tests both commercial kits and "homemade" tests have appeared on the market. As big service laboratory Biopticka laboratory wanted to choose the best quality test to offer our cooperating obstetricians, so we paid attention to the significant differences in quality of available non-

invasive tests (NIPT) from molecular and clinical perspective.

Materials and Methods: We implemented and validated Natera's Panorama® test as a constellation partner laboratory, we have been running in-house service since February 2017.

Results and Conclusions: We would like to share our over two years' experience with Panorama testing, providing testing for over 3000 patients in-house. Results confirm qualitative benefits, but also some drawbacks of the targeted single nucleotide polymorphism (SNP) based NIPT approach of the Panorama® testing.

M. Putzova: None. **L. Sprdlikova:** None. **M. Hasch:** None. **B. Gomolcakova:** None. **M. Michal:** None.

E-P01.11
Experience after 3 years of NIPT (Non-Invasive Prenatal Testing)

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From the blood of pregnant women with an increased risk of fetal aneuploidy the PraenaTest® (Lifecodexx) determines trisomies 21, 18 and 13 as well as gonosomal aneuploidies (Turner's, Triple X, Klinefelter's and XYY syndromes) from 10th week of pregnancy. We have summarized the clinical experience over the past 3 years (>10000 analysis) regarding the performance of the NIPT for fetal aneuploidy. In total, 91.61% of the analyses showed inconspicuous results (i.e. Z-score within the normal range) after the initial analysis. 5.91% of the analyses were not assessable because the Z-score was within the non-evaluable range (autosomes 13, 18, 21: 2.32%; gonosomes: 3.59%). Here, analysis of a second test tube usually showed a conclusive result for chromosomes 13, 18, 21; whereas the result for gonosomes often remained not assessable. A total of 1.22% of the ordered tests were cancelled for technical reasons (failed quality criteria). Here, a repetition using a second test tube resolved most of the cases. Finally, 0.16% remained without results due to failed quality criteria. In summary, 1.28% of analyses revealed a high-risk for chromosomal aneuploidy (i.e. Z-scores beyond the normal range). NIPT results that are inconsistent with the results of diagnostic prenatal tests (CVS, amniocentesis) were most commonly reported for gonosomal aneuploidies. In summary, PraenaTest® is a screening test which needs to be offered in the context of genetic counselling. It does not replace the precision achieved with a diagnostic test and it should not replace the differentiated fetal ultrasound examination of the fetus.

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E-P01.12
Diagnosis of Noonan syndrome for an aborted fetus by whole genome sequencing

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Introduction: A Lymphatic Malformation (LM)/cystic hygroma is a fluid-filled sac that results from the abnormal development of lymphatic vascular system. When identified in a fetus, it may increase the risk for miscarriage or fetal death. It is common in infants with genetic diseases, such as Turner syndrome, Down syndrome or Noonan syndrome. In many cases, the cause is still unknown.

Methods: we performed low and high coverage whole genome sequencing (WGS) for an aborted fetus, who was discovered a LM at the neck via ultrasound at the 11th week of pregnancy.

Results: The LM was 21X8 mm when firstly detected. It developed as large as 24X12 mm one week later and hydrops within the baby's body was observed. We did not identify abnormal chromosome using low coverage sequencing. Next, we analyzed the high coverage WGS data and detected a heterozygous missense mutation c.182A>C (p.Asp61Ala) on the gene *PTPN11*, which is known to cause a large fraction of the cases of Noonan syndrome. This mutation is a reported pathogenic mutation for the autosomal dominant disorder. Furthermore, it was validated as a *de novo* mutation after sanger sequencing of the fetus and parents.

Conclusion: We conclude that the LM and hydrops of the fetus are part of the features of Noonan syndrome, caused by a *de novo* mutation on the pathogenic site of the *PTPN11*. Our findings provide potential application of WGS to clinical diagnosis and it could also contribute to the genetic counselling of couples with miscarriage history.

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E-P01.13
Thee Good the Bad and the Ugly of Direct to Consumer Genetic Tests

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The author presents 2 cases of paternity search performed using Direct to Consumer Tests (DCT) from the searcher 's point of view for one family and from the father's side in another family.

The first case presents to the clinic with results of his abnormal spermogram from which he is convinced that he may not be the father of his previous born son. He went on to present the DCT result of his 2 further born children, his wife, his previous born son. The discussion was about the understanding of these tests results, detailed presentation of the results data, the validity of commercial genetic tests and the psychological reasons for these tests.

The second family presents with a man whose 2 cousins who had previously performed DCTs and were later contacted by email about an unknown adopted individual claiming he had matched with them as possible cousins using the DCT databank family search tool. The man at first had not answered these emails from his cousins he thought were a joke until they called him informing him he may have been the father of this unknown individual from temporal and geographical evidence. The reaction, action and results of these will be presented in details. These cases are iconic examples of what geneticists may expect to be confronted with as the use of DCT are becoming commonplace and with their various impact on individuals and families.

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E-P01.14
Application of whole-exome sequencing in daily practice: reducing the cost, diagnostic odyssey, increasing the diagnostic rate

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In daily clinical practice of inherited genetic disorders, wide spectrum and overlapping phenotypes beside the locus and allelic heterogeneity make it challenging to identify the genetic cause of the disorders. Usually it is difficult for the practicing clinician to visit a patient, identify a candidate gene or mutation, and test for a single genetic disorder. Using advanced NGS based methods like whole-exome sequencing (WES) made it feasible to study all coding genes in a patient. This study was performed in Kawsar human genetics research center and 397 sample was recruited from 230 consecutive families with at least one affected case suspected to inherited Mendelian disorders including developmental delay/intellectual disability, inherited neuromuscular disease, hearing impairment and inborn error of metabolism. A pathogenic or likely pathogenic variant was found in 122 patients (48.69%). Variant of unknown significance (VOUS) was also found in 48 cases (20.87%). Identified pathogenic variants were related to 42 different genes, mostly were novel that was not reported before. Instead of being still expensive with limitation in diagnosis of repeat expansion disorder (e.g., spinocerebellar ataxia), mutation in a non-coding region (e.g., facioscapulohumeral muscular dystrophy) or large deletion duplication, WES is more reasonable against sequential gene testing that is more costly and time-consuming. It seems to be better to assess phenotypes with broad phenotypic variability and overlap of many potential genetic causes with WES and not reserve it as a last resort.

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E-P01.15
Indication and outcome of prenatal samples tested in the laboratory of the Department of clinical genetics Teaching hospital Olomouc, Czech Republic in 2017

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Introduction: We set out to analyse the reasons for an invasive procedure and the outcome of the cytogenetic testing of the samples tested in our laboratory in 2017.

Materials and methods: Retrospective analysis of the samples examined in the cytogenetic laboratory of the teaching hospital Olomouc, Czech Republic, which serves the hospital and other privates prenatal centres in the city.

Results: 195 samples were tested, 142 form hospital patients, 53 from external patients. Of the hospital patients 70% were tested because of a positive result of combined first trimester screening, 18% for congenital abnormality. CVS was performed in 46% of the external samples and in

56% of hospital samples. In the CVS group only 3 procedures were needed per diagnosis in the external group compared to 11 in the hospital group. In the amniocentesis group 30 procedures were needed per diagnosis in the external group compared to 15 in the hospital group.

Conclusion: In our sample the positive predictive value of prenatal testing differed greatly between the hospital and the external providers. This may reflect the diverse reasons for testing and the fact that no standardized procedure exists for first trimester combined screening in the Czech Republic.

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E-P01.16
Revealing hidden phenotypes in the Mendelian disorders: teratozoospermia due to *KRT9* pathogenic variant?

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Introduction: Teratozoospermia is a condition characterized by presence of sperm with abnormal morphology that affects fertility in males. In the majority of cases the causes are unknown, however recent studies suggest mutations in several genes to be implicated. In vitro fertilization has high success rate in teratozoospermic men, and natural conceptions have also been described.

Materials and methods: Here we describe a 38-year-old male patient, with difficulties conceiving due to severe teratozoospermia (<1% normal, predominating spermatozoa with amorphous head changes and excess residual cytoplasm in the midpiece) with coexisting epidermolytic palmoplantar keratodermia (EPPK) and hearing impairment. Genetic analyses included clinical exome sequencing using Illumina TruSight One gene panel and Sanger sequencing for familial testing.

Results: *De novo* heterozygous mutation in *KRT9* gene (NM_000226.3:c.487C>T) and compound heterozygosity for *GJB2* mutations (NM_004004.5:c.[35delG];[269T>C]), known to cause EPPK and hearing impairment, respectively, were identified. No pathogenic mutation was found in the genes associated with teratozoospermia. Studies have shown that *KRT9* gene is expressed in developing spermatids and its loss of function causes teratozoospermia and reduced fertility in mutated mouse. Thus, we believe that *KRT9* mutation in our patient might be responsible both for EPPK and teratozoospermia. Since *KRT9* c.487C>T mutation has been described in several generations of EPPK families, it may have an incomplete penetrance for male infertility.

Conclusions: Our study suggests extension of *KRT9* associated phenotype. Fertility status evaluation and semen analysis in other patients with *KRT9* mutations may help to clarify the role of *KRT9* gene on sperm morphology and male fertility.

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E-P01.17
Risk evaluation of thrombophilia in pregnancy

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Introduction: Thrombophilia's are hereditary or acquired conditions that predispose patients to thrombosis and it can increase the risk of venous or arterial thrombosis. The study wants to determinate the risk of thrombophilia in pregnancy and the importance of correct treatment administration in time. Materials and Methods: This is a retrospective study, we have included 58 pregnant women with thrombophilia. The clinical information is containing the following: age, thrombosis type and location, indication and timing of testing, anticoagulation therapy at the time of testing. There were measured four events: un-provoked/ provoked venous/ arterial thrombosis (risk factors), venous thromboembolism, pregnancy morbidity and recurrent pregnancy losses.

Results: The median age observed was 31 years (19–44 years). From the total range, 12% of them showed thrombosis events in antecedents, 68% had family history, 75% had more than 3 lost pregnancies, 3% showed infertility. Identifying women with risk of venous thrombosis has become a priority and the identification of thrombophilia markers has grown steadily. Inherited risk factors for venous thrombosis include deficiencies of the natural anticoagulants: antithrombin III, protein C and protein S. The patients possess genetic polymorphisms such: factor V Leyden mutation (33%), prothrombin mutation G20210A(27%), plasminogen activator inhibitor (17%), two polymorphism of the methylenetetrahydrofolate reductase gene C677T and A1298C(23%). 72% of pregnant patients with thrombophilia where categorized with high risk of thrombophilia, that triggered the administration of low molecular weight heparin.

Conclusions: The tests should be carried out in situations with clinical suspicion of thrombophilia, because the treatment decision in pregnancy could be different.

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

E-P02.01
A case report of a boy with congenital nonsyndromic deafness: Identification of a homozygous missense variant in the *OTOF* gene

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Introduction: The diagnosis of *OTOF*-related deafness is confirmed by identification of biallelic deafness-related variants in *OTOF*, the gene encoding the protein otoferlin, on chromosome 2p23.3. In this report we will present the clinical characteristics and molecular data of the boy with prelingual nonsyndromic sensorineural hearing loss in whom we identified a homozygous missense variant in the *OTOF* gene.

Materials and Methods: An specific allele-specific polymerase chain reaction (ARMS-PCR) was used as the first trial test for the detection of the c.35delG mutation in the *GJB2* gene. Next generation sequencing (NGS) of genomic DNA was performed by Sistemas Genomicos® (Oto-GeneSGKit®). Sample sequencing was done on the Illumina HiSeq®/MiSeq® platforms. GeneSystem® and Variant studio (Illumina) software were used for the analysis and the interpretation of the NGS data.

Results: ARMS-PCR for the detection of c.35delG mutation relieved the presence of a heterozygous frameshift variant in the *GJB2* gene in the proband, which was inherited from the mother. Furthermore, next-generation sequencing detected a homozygous missense variant c.2464C>T of uncertain significance in the *OTOF* gene. The same variant c.2464C>T, but in a heterozygous state, was found in his parents and sister.

Conclusions: The genetic heterogeneity underlying nonsyndromic deafness complicates genetic testing for deafness in most populations. Our case highlights the possibility that the above-mentioned variant could be the cause of prelingual nonsyndromic sensorineural hearing loss. To date, two more deaf patients (siblings) with this variant in a homozygous form in the *OTOF* gene have been reported.

A. Golub: None. **A. Erjavec kerget:** None. **B. Zagradinik:** None. **J. Rebol:** None. **D. Krgović:** None. **N. Kokalj Vokač:** None.

E-P02.05
Novel LCA5 mutation causes Retinitis pigmentosa

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Introduction: A consanguineous Bedouin family presented with a phenotype of Leber Congenital Amaurosis (LCA) affecting an individual at the latest generation in an apparent recessive mode of inheritance.

Materials and Methods: Whole exome sequencing data of an affected individual were analyzed and filtered for known benign variants using our in-house databases along with open access databases (1000 genomes, NHLBI ESP, ExAC).

Results: The analysis yielded several candidate variants in genes previously associated with various ocular disorders. Among candidate variants, a novel homozygous missense p.K391* mutation in Leber Congenital Amaurosis 5 (LCA5) was the only one in a gene previously associated with LCA. The mutation was verified by Sanger sequencing and was found to be fully segregate in the affected kindred as expected for recessive heredity. Furthermore, the mutation was not found in 100 healthy ethnically-matched controls. In-silico analysis of the p.K391* variant showed that it is likely to have a deleterious effect on the mature protein.

Conclusions: Mutations in LCA5 were previously described as causative for conditions such as Retinitis pigmentosa and Leber Congenital Amaurosis. Our data suggest that the novel LCA5 homozygous mutation is the cause for the autosomal recessive, isolated, inherited LCA in this kindred.

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E-P02.06
Postlingual deafness in Eveno-Bytantaysky National

District of the Sakha Republic (Eastern Siberia, Russia): audiological and clinical-genealogical analysis

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In this paper we present for the first time the results of the audiological and clinical-genealogical research of the population of settlements Batagai-Alyta and Kustur of the Eveno-Bytantaisky National District (ulus) of the Sakha Republic (Eastern Siberia, Russia) for studying the postlingual form of deafness of unknown etiology firstly identified by us earlier in three Evens. As a result of an audiological examination of 72 people, 10 patients from 6 nuclear families who met the criteria of postlingual form of hearing loss were found. The segregation analysis carried out in these families confirmed the autosomal recessive type of inheritance of this form of postlingual hearing loss. The distant relationship of the examined patients with postlingual hearing loss living in two villages of the Eveno-Bytantaisky National District of the Sakha Republic can indicate to the role of the founder effect in the local prevalence of this pathology. The results of this study and obtained expedition material will be the basis for further study of the molecular genetic etiology of this form of deafness and the discovering of mechanisms of its accumulation in this region of Sakha Republic. This study was supported by the Ministry of Science and Higher Education of the Russian Federation (Grant #6.1766.2017, the Russian Foundation for Basic Research (Grants #17-29-06-016_ofi_m, #18-015-00212_A, #18-013-00738_A, 18-05-600035_Arctica) and the program for support of the bioresource collections of FASO of Russia "The Genome of Yakutia" YSC CMP (BRK 0556-2017-0003).

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

E-P03.03
Clinical characteristics of patients referred for *HNF1B* testing - Polish population study

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Introduction: Patients with heterozygous mutations of *HNF1B* typically develop renal cysts and diabetes syndrome (RCAD). However, the extent of pancreatic and renal involvement may vary, and additional manifestations often broaden the phenotypic spectrum. We analyzed probands referred for *HNF1B* testing, comparing patients with, and without genetic diagnosis.

Methods: Probands tested for *HNF1B* in years 2005-2018 using Sanger sequencing, multiplex ligation-dependent probe amplification, and next generation sequencing, were retrieved from the Polish Monogenic Diabetes Registry. A structured medical interview was performed with all individuals and their physicians. Patients without clear indication for *HNF1B* testing at referral (diabetes and renal abnormalities or family history for those conditions) were excluded. All genetic findings were reassessed according to ACMG criteria.

Results: The study included 35 unrelated probands, among which 12 harbored a pathogenic or likely-pathogenic variant in *HNF1B* (five whole-gene deletions, one indel, five SNVs), and one a variant of uncertain significance. Among the 22 patients negative for *HNF1B*, four had non-*HNF1B* findings in *GCK*, *PKD1*, *KCNJ11* and *HNF4A*. Presence of polycystic kidneys (OR = 6, 95% CI:1.23–29.45) and pancreatic abnormalities (OR = 15, 95%CI:1.49–151.30) best discriminated the HNF1B-positive cases from the negative ones.

Conclusions: Patients referred for *HNF1B* testing present very heterogenous phenotypes. Despite suggestive characteristics, many do not harbor mutations in HNF1B

warranting further investigations into the genetic basis of the RCAD syndrome.

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E-P03.05

Copy number variation analysis in 164 patients with lower urinary tract obstruction

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Introduction: Congenital lower urinary tract obstruction (LUTO) is caused by anatomical blockage of the bladder outflow tract or by functional impairment of urinary voiding. Associated with early-onset oligohydramnios and renal disease, it is a major cause for childhood renal insufficiency and dialysis. Recent studies indicated the importance of copy number variations (CNVs) as genetic risk factor for LUTO. To systematically identify rare disease-causing CNVs, we performed a genome-wide CNV analysis in LUTO patients.

Methods: An array-based molecular karyotyping was performed in 164 LUTO patients using Illumina GSA1.0. CNVs were calculated from raw intensity data. In order to exclude common CNVs a filter has been developed filtering each CNV against 4168 healthy controls. CNV overlapping features were annotated using AnnotSV. Only CNVs containing gene- or promotor-coding regions were included. In a manual step each CNV is currently filtered for frequency in the Database of Genetic Variants.

Results: In the genome-wide analysis, we identified over 900 possible disease-causing CNVs. The application of additional filter criteria is ongoing in order to prioritize possible disease-causing CNVs/candidate genes. In one abort a deletion in chr2:143,581,413–150,270,689 (6Mb) was identified causing Mowat-Wilson Syndrome.

Conclusions: After validation and segregation of the possible disease-causing CNVs of the genome-wide analysis, a re-sequencing of candidate genes in a larger cohort could be suggested to further investigate the impact of variants in these genes on LUTO. Further our study suggests that prenatal CNV testing in non-isolated LUTO should be warranted.

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E-P03.06

Maturity onset diabetes of the young type 3and pregnancy: Spontaneous hypoglycaemia and different pregnancy courses in two sisters with an*HNF1a*mutation

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Introduction: Diabetes is a known risk factor associated with a number of adverse pregnancy outcomes. Consequently, well-regulated pre-conception blood glucose levels and closely monitored glycaemic control during pregnancy are considered pivotal for optimal foetal outcome and maternal health. Maturity onset diabetes of the young (MODY) comprises a group of monogenetic dominantly inherited types of diabetes. This paper aims to describe the phenotypes of two pregnancies in two sisters heterozygous for the c.526C>T-mutation in *HNF1a*.

Materials and Methods: Clinical assessment during pregnancies included biochemical measurements, biometrics, blood pressure, and ultrasonic assessments with regards to maternal and foetal health. Data were retrieved from the sisters' medical records.

Results: Pre-gestational HbA1c-levels were elevated in both sisters, following a steady decline throughout the first half of both pregnancies. Likewise, both sisters initially suffered low fasting glucose levels in early pregnancy.

Glycaemic control in Sister A deteriorated during the pregnancy despite treatment with sulfonylurea and later insulin, with glucose levels stabilizing shortly after delivery. Sister A suffered from preeclampsia, and delivered a macrosomic child by vaginal delivery at gestational week 34+4. Sister B retained fasting hypoglycaemia without medicinal treatment of the diabetes throughout the pregnancy. Sister B delivered a child after induction at gestational week 35+6 due to gestational hypertension.

Conclusion: Two sisters with MODY3 due to the same *HNF1a* mutation presented very different pregnancy courses and glycaemic control during pregnancy. Spontaneous hypoglycaemia in early pregnancy may complicate the management of diabetes in MODY3 patients.

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E-P03.08
The effect of exogenous and genetic factors on the development of vitamin D deficiency in patients with cystic fibrosis

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Objective: To investigate the level of Vitamin D in Cystic Fibrosis (CF) children in the three regions of the Russian Federation with taking due to exogenous and genetic factors. Materials and methods: 158 CF patients were examined during the winter period: 73 from Moscow (51 - CF, 22 healthy), 45 from Krasnoyarsk (29 - CF, 16 healthy), 40 from Stavropol (20 - CF, 20 - healthy). Polymorphic variants *CYP2C9*3, CYP2C9*2, CYP2D6*4, CYP3A4*3, CYP3A4*1B* were investigated. The synthesis of endogenous antimicrobial peptides was determined by enzyme immunoassay using Hycultbiotech kits. Results. Significantly lower serum Vitamin D levels were determined in CF patients from 3 regions, compared with healthy controls ($p = 0.003$). With increasing age, the content of vitamin D in the serum of patients with CF was reduced ($p < 0.01$). The lowest rates of Vitamin D were recorded in the Stavropol region ($p = 0.001$). No relationship was found between the carrier of polymorphic variants of genes of the first phase of xenobiotic biotransformation and the level of Vitamin D in CF patients. The level of HNP1-3 was significantly higher in CF children compared with healthy controls ($p = 0.017$) and does not depend on the content of Vitamin D. Conclusion. The availability of vitamin D depended on the dose of vitamin D. A low level of vitamin D was registered in patients with VC less than 80%, in those with cirrhosis of the liver and those taking glucocorticoid drugs. The work was carried out within the framework of the state task.

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

E-P04.01
A genome-wide association study of bisphosphonate-associated atypical femoral fracture

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Introduction: Atypical femoral fracture is an adverse effect of bisphosphonate drugs that are used to prevent the loss of bone density in osteoporosis and metastatic bone disease. The mechanism of this adverse effect is unclear, but a genetic predisposition has been suggested. We performed a genome-wide association study with the aim to identify common genetic variants predisposing to atypical femoral fracture associated with bisphosphonates.

Materials and Methods: Cases were recruited mainly through reports to the Swedish Medical Products Agency. Cases without a current diagnosis of cancer ($n = 51$) were compared with population controls ($n = 4891$), and with bisphosphonate-treated controls without a current diagnosis of cancer ($n = 324$). Single nucleotide polymorphisms (SNPs) were imputed using the haplotype reference consortium panel. The genome-wide significance threshold was $p < 5x10^{8}$.

Results: Bisphosphonate-associated atypical femoral fracture was associated with four isolated SNPs: rs7729897 upstream of *NR3C1*, OR 10.27, $p = 4.00x10^{-10}$; rs11465606 intronic in *IL18R1*, OR = 6.15, $p = 7.13x10^{-9}$; rs145787127 intronic in *NTN1*, OR = 7.37, $p = 3.08x10^{-8}$; and rs144094653 close to *TUBB8P5*, OR = 7.68, $p = 4.20x10^{-8}$. Variants of *NR3C1* and *NTN1* have previously been associated with decreased bone mineral density and osteoporosis, respectively. When cases were compared with bisphosphonate-treated controls, no statistically significant association remained.

Conclusions: We found no evidence of a common genetic predisposition to bisphosphonate-associated atypical femoral fracture. Further studies with larger sample sizes, and whole genome sequencing studies to identify possible rare risk variants, are warranted.

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E-P04.02
Regenerative approach for repairing large odontogenic cystic defects in two genetic disorders

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Introduction: Odontogenic cysts arise from tissue(s) that are involved in tooth development and usually cause a variable degree of bone resorption. Following the cyst enucleation, whether or not to fill the resultant bone defect with bone substitute depends mainly on the size of the defect. In this study, we present two cases with large odontogenic cystic lesions that have been treated via a regenerative approach. Material and methods: The first case was a 16 years old girl diagnosed with Xeroderma Pigmentosum (XP) and the second case was a 48 years old female had diabetes mellitus type 2, systemic lupus erythromatosus and under cortisone treatment. Histopathological examination revealed a cyst from odontogenic origin either dentigerous or radicular. Autologous bone marrow mononuclear cells (BMMNCs), nano-hydroxyapatite, gelatin sponge and autologous platelet-rich fibrin (PRF) was used to fill the resultant bony defect.

Results: Radiographic assessment was performed after six months showed bone regeneration with few trabeculations. Regular follow-up last for three years and radiographic evaluation showed successful and complete bone regeneration with normal trabeculations and no sign of cyst recurrence.

Conclusions: This study proved that the regenerative approach led to satisfactory healing of large odontogenic cysts defects and it positively induced bone regeneration, regardless of the type of genetic disorder.

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E-P04.03
Genomic analyses in the Lebanese population identify risk loci for cleft lip and palate

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Introduction: Cleft lip and palate is a common complex developmental disorder that presents with medical, socio-economic and psychological problems. The aim of the study was to identify known and/or novel genes and gene variants that play a role in the susceptibility to cleft lip and palate in the Lebanese population.

Materials and Methods: Seven unrelated families were chosen based on positive consanguinity history, and/ or positive family history for isolated as well as syndromic clefts and tooth agenesis. Whole exome sequencing was performed on 30 members and comprehensive filtering strategies were carried out on the single nucleotide variant and indel files. *In silico* tools were used to model and predict potential causality of the variants having a minor allele frequency of ≤ 2%.

Results: Ten variants showed a co-segregation with the condition in our families and were thus shortlisted. Variants included missense and nonsense changes in the *Notch 2* (rs141935585), *CFTR* (rs377319489), *PGAP3* (rs761733666), and *MID2* (rs763059099) genes.

Conclusions: Replication studies will help assess the shortlisted variants in populations of different ethnic origins. In parallel, we are analyzing one variant using functional studies. This would help us understand the physiological mechanism behind the failure of proper fusion at the level of embryological facial buds. (http://sas.lau.edu.lb/natural-sciences/people/michella-ghassibe-sabbagh.php) (michella.sabbagh@lau.edu.lb)

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E-P04.04

Splicing mutation (c.1155 + 1G>C) in the COL1A1 gene in a Romanian patient with osteogenesis imperfecta followed by prenatal diagnosis

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Osteogenesis imperfecta (OI), (OMIM®: 120150) also known as brittle bone disease, is a rare heterogeneous group of inherited disorders. The purpose of this study is to report one patient, with OI type I in a Romanian family with an RNA-splicing mutation in COL1A1 gene. The 37 years old patient was clinically diagnosed at birth with OI type I, presenting right clavicle fracture. Since birth, he had 60 fractures, mostly lower limbs fractures, consequently now has short stature (1.36 m). The patient has blue sclerae, but no dentinogenesis imperfecta and no degree of hearing loss.

Methods: For the patient, a Clinical Whole Exome Sequencing test was performed focusing on 3583 OMIM disease genes with target region capture followed by Next Generation Sequencing. NGS testing was followed by prenatal diagnosis. His wife was pregnant, amniocentesis was performed, amniocytes were cultured, then DNA extraction followed by targeted sequencing was performed on both DNA strands of the relevant COL1A1 region.

Results: Direct DNA sequencing analysis of COL1A1 gene revealed a splicing mutation (c.1155+1G>C or IVS17 +1G>C) in heterozygous state. The c.1155+1G>C mutation has been reported for its pathogenicity being present in the LOVD database with 7 entries, all are substitutions within intron 17, classified as pathogenic splice-site variants. The fetus is not a carrier of this mutation.

Conclusion: Each reported case is important because it helps to know the pathogenic gene mutation in Romanian patients with OI and the detailed molecular and clinical features will be useful for exploring phenotype-genotype correlations.

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E-P04.05

Heritable disorders of connective tissue: diagnostic approach and coordination of care in 10 unrelated cases

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Introduction: There are more than 450 heritable disorders of connective tissue and represent a heterogeneous group that affect one or another of the primary elements of the connective tissues, characterized by abnormalities in skeletal tissues muscle, skin, the cardiovascular system, eyes, lungs and may interfere with normal growth and development. The aim of our present study was to assess genetic diagnosis of suspected patients by connective tissue disorders and to establish the nature of the management with a personalized therapeutical program.

Methodology: Full work-up was done to the patients presenting with a phenotype involving connective tissue: Ehlers-Danlos syndrome, Marfan syndrome, cutis laxa spectrum, melorheostosis with osteopoikilosis, different congenital collagen disorders. Each patient was confirmed with molecular investigation of the responsible gene. Targeted monogenic diagnosis, gene panel testing or Whole Exome Sequencing technique have been performed, depending on clinical suspicion of the case.

Results: In our group known or new variants have been described involving the genes: *EFEMP2, TNXB, PYCR1, FBN1, LEMD3, COL5A1, COL4A1, COL4A4, COL10A1*. The mutations of these genes can lead to various diseases with clinical heterogeneity, with secondary physical discomfort, medical complications and social repercussions. Critical areas of clinical management comprised pain, cardiovascular and respiratory issues, fatigue and dysautonomia, bone fragility, skin and soft tissue fragility. Physical therapy, psychological support and reproductive concerns represented major issues for care of these patients and their families.

Conclusions: Understanding and appreciation for the medical issues in each diagnosed disorder improve the quality of life and life span of these individuals.

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E-P04.07

First case of non-lethal variant in Fibrochondrogenesis case in Kuwait

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Background: Fibrochondrogenesis (FCG) is rare lethal chondrodysplasia disorder characterised by recessively inherited disorder and multiple skeletal anomalies [1-3,5]. To date, there are 26 reported cases in the literature, where only few reported cases survived after first year of life [4,5]. Here, we are reporting a case of FCG who survived beyond neonatal and even first year of life with unusually mild-to-moderate sensory-neural deafness. To our knowledge, this is the first case of FCG in Kuwait.

Material and methods: we are reporting an girl whom first came to genetic clinic at the age of 18-months presenting with short limbs, facial dysmorphism and cleft palate. Skeletal survey showed multiple skeletal anomalies including rhizomelic shortening, possible hypoplastic pelvic bones, displaced and adducted thumb B/L. Both Kniest and FCG were consistent based on both clinical and radiological features. However, FCG was initially excluded because it is known to be lethal. As a result *COL2A1* gene analysis to rule-out Kniest syndrome was requested, which surprisingly revealed no detectable mutations. After that, skeletal dysplasia gene panel revealed novel likely pathogenic variant (c.2323G>C) in *COL11A1* gene in homozygous state.

Result: such a surprising genetic result is consistent with the genotype-phenotype contrary to the lethality nature of FCG. Furthermore, both parents are found to be heterozygous carriers further supporting the pathogenicity of the detected variant.

Conclusion: although FCG is a lethal skeletal dysplasia; however, we detected non-lethal likely pathogenic variant in our patient.

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E-P04.10
Mandibuloacral dysplasia type A; an oro-dental perspective

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Background: Mandibuloacral dysplasia type A (MAD A) (OMIM #248370) is a progeria laminopathy. We report the oro-dental manifestations in five patients with MAD A from three unrelated families.

Subjects and Methods: Patients were recruited from the outpatient clinics of genodermatoses and oro-dental genetics, NRC, Egypt. After acquiring the informed consents; pedigrees were constructed, phenotyping was performed, dental panoramic radiographs were taken and molecular studies were performed to confirm the diagnosis.

Results: The first family had 3 siblings, 1 female and 2 males. The second and third families had a male and a female respectively. All patients are the result of consanguineous marriages. Their ages ranged from 8–17 years. Oro-dentally, all five patients showed microstomia and severe crowding leading to excessive proclination of the anterior incisors and incompetence of the lips which increased as the patients aged. The repercussions of crowding from caries and calculus formation also increased by aging. Dental panoramas showed accentuated mandibular angles & severe micrognathia. Bent roots were present in 4 of the cases. The mandibular ramus was severely short in 2 cases and it was absent completely in 3- from two families- with subsequent absence of the condyle and coronoid processes. Acro-osteolysis of the chin was evident in the two elder siblings.

Conclusion: This study extends the phenotypic spectrum of MAD A with the absent ramus, condyle and coronoid as an additional feature found in three of the cases herein. This finding also supports the suggested role of *LMNA gene* in intramembranous ossification of bone.

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E-P04.12
Intranasal desmopressin treatment for massive subcutaneous hematoma in five patients with musculocontractural Ehlers-Danlos syndrome

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Musculocontractural Ehlers-Danlos syndrome (mcEDS) is a recently delineated type of Ehlers-Danlos syndrome (EDS), associated with impaired synthesis of dermatan sulfate caused by mutations in *CHST14* or *DSE*. Massive subcutaneous hematoma is one of the most serious complications in mcEDS, occurring with mild traumas,

spreading acutely with severe pain, and sometimes resulting in hemorrhagic shock. Intranasal or intravenous desmopressin treatment is used for patients with mild hemophilia and von Willebrand disease through increased activities in factor VIII and von Willebrand factor. The treatment was also described to be useful for management of bleeding in patients with various types of EDS. We report experiences intranasal desmopressin treatment (150μg/episode) on five patients (1 male, 4 females; the mean age 18.2 years) in Shinshu University Hospital. Clinical information was retrospectively reviewed from medical records of these patients. The mean age of the first episode of massive subcutaneous hematoma was 9 years (range, 3 to 15). Surgical drainage was required in three and blood transfusion in two. Elevated activities of factor VIII and von Willebrand factor were measured in all after administration of intranasal desmopressin. Four continue the treatment without adverse effects, whereas one child discontinued it because of transient numbness in an arm. The treatment was realized by the patients and their families to suppress growth of hematoma, shorten the duration of suffering, and relieve psychological burden about the risk of massive subcutaneous hematoma. Intranasal desmopressin treatment could be a preventive and therapeutic option for massive subcutaneous hematoma in patients with mcEDS.

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E-P04.16

A compound heterozygous Egyptian patient with a mild form of Papillion LeFevre syndrome

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Introduction: Papillon-Lefèvre syndrome (PLS) is a rare autosomal recessive genodermatosis, characterized by palmoplantar hyperkeratosis combined with early-onset severe periodontitis. On the other hand, aggressive periodontitis (AgP) is an early-onset rapid progressive destructive disease that affects the periodontal ligament and alveolar bone but without dermal manifestations. Mutations in CTSC gene were found to be responsible for PLS and in some cases for AgP. Here, we present a case with PLS that was initially confused with aggressive periodontitis and carries two different mutations in the CTSC gene on each chromosome.

Patient and methods: A 14-year-old girl, was referred to our Oro-dental Genetics clinic. She was born to apparently healthy non-consanguineous parents. Her deciduous teeth erupted and exfoliated normally. Although her permanent teeth were all present, mobility of some of the teeth and mild gingivitis were present. Panoramic radiographs showed mild generalized horizontal bone loss, only severe at the areas of first molars and anterior teeth in both jaws. Moreover, mild dryness and fissuring of the skin of the palms and soles were observed. After obtaining written consent, peripheral blood samples were obtained from the patient and her parents. Sequencing of the coding exons of CTSC gene was performed.

Results: Despite the mild clinical features of PLS, compound heterozygous mutations of CTSC gene were identified on exon 7 (c.935A>G and c.1234A>G) in the paternal and maternal chromosomes, respectively. The parents were found to be heterozygous carriers without any clinical feature of PLS.

Conclusion: Some forms of PLS can be confused with aggressive periodontitis

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E-P04.18

Variable familial expression of spondylometaphyseal dysplasia with coxa vara& a novel *FN1* mutation

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Introduction: Spondylometaphyseal dysplasias (SMDs) comprise a diverse group of skeletal dysplasias and often manifest as short stature, growth-plate irregularities, and vertebral anomalies. One such condition is SMD with "corner fractures" (OMIM #184255). These individuals generally show development of coxa vara, scoliosis and triangular ossification centers at the edges of metaphyses that simulate fractures. To date 16 patients with SMD and biallelic Fibronectin (*FN1*) mutations have been reported. The majority had a substitution of a cysteine in the N-terminal assembly region.

Methods: Analysis of candidate genes by massive parallel sequencing (TruSight™ One Panel, NextSeq Illumina) and data analysis by SeqNext (JSI).

Results: We report a family with a novel missense mutation in *FN1*:c.341G>C (p.Arg114Pro) inherited from the mother. The girl, born at term BW 1,895kg (−3, 03 SD), L 44cm (−2,83SD), was first evaluated for short stature and

developing waddling gait at 7 years. Radiographic features were compatible with a bilateral "perthes like" hip dysplasia, coxa vara, flattened epiphysis with corner fractures, abnormal vertebrae with end-plate irregularities. Height at 8,4years 113,5cm (−3,5SD).

The brother was born preterm at 33/3 weeks with normal birth measurements, normal development. At 9 years he developed an abnormal gait caused by unilateral coxa vara. Height was 127,6cm (P10).

The mother was evaluated in her childhood with short stature and coxa vara. Final height was 149cm. In adulthood she developed painful osteoarthritis and osteonecrosis affecting mainly knees and ankles.

Conclusion: The report expands the clinical phenotype and demonstrates familial variability concerning onset and severity of symptoms.

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E-P05
Cardiovascular disorders

E-P05.02
PITX2 and *NEURL1* SNP polymorphisms in Hungarian atrial fibrillation patients determined by quantitative real-time PCR and melting curve analysis

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Introduction: Atrial fibrillation (AF) is the most common cardiac arrhythmia affecting 1–2% of the general population. The aim of our study was to investigate whether the rs2595104 SNP located in the enchancer of the *PITX2* at 4q25 region and rs6584555 SNP in an intronic region of *NEURL1* gene on chromosome 10 are associated with AF in a Caucasian population. PITX2 and NEURL1 regulate the expression profile of ion transporters and changes in action potential duration so reduced expression of both proteins in patients could increase AF susceptibility by AP duration shortening.

Materials and Methods: We performed a protein-protein network analysis to assess functional connection among the protein products. We genotyped DNA samples of 76 AF patients and 77 healthy controls using quantitative real-time PCR followed by melting curve analysis.

Results: The frequency of minor *A* allele (rs2595104) of *PITX2* was 0.38 and 0.44 in the control group and in AF patients, respectively. There was no significant difference in allele and genotype distribution ($p = 0.52$). The log additive odds ratio is 1.235 (C.I. = 0.783–1.947; $p = 0.363$). The frequency of minor *C* allele (rs6584555) of *NEURL1* was 0.22 in the control group and 0.23 in AF patients. In allele and genotype distribution we could not find any significant difference ($p = 0.92$). The log additive odds ratio is 1,056 (C.I. = 0.618–1.805; p = 0,842).

Conclusion: We did not find significant association of SNP rs2595104 and rs6584555 with AF. The network analysis showed that PITX2 and NEURL1 are connected indirectly via two intearctors take part in Wnt and Notch signaling pathways.

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E-P05.03
Familial association of both molecularly confirmed type 1 Neurofibromatosis and Brugada syndrome

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We present a family in which segregates the inherited nonsense mutation [c. 3946C > T (p.Arg1316*)] in the *SCN5A* gene in association with Brugada syndrome (BrS). In the same family segregates the frameshift mutation [c.7686delG (p.Ile2563fsX40)] in the *NF1* gene as well, associated with type 1 neurofibromatosis (NF1). This genetic association, reported for the first time ever, might identify a subset of NF1 patients at higher risk of sudden cardiac death. We think that those patients require a careful arrhythmologic counselling in addition to clinical evaluation performed for type 1 Neurofibromatosis. Our case series highlights the importance of genetic testing not only to confirm a pathology but also to identify asymptomatic family members. They can need further clinical examinations and preventive interventions, as well as to be advised about the possibility of avoiding recurrence risk with medically assisted reproduction. Further studies are required to clarify the relationship between molecularly confirmed NF1 and arrhythmias.

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E-P05.05
TCF21 rs12190287 and GATA4 rs804280 in pediatric patients with Congenital Heart Disease

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Introduction: Single nucleotide polymorphisms (SNPs) of GATA4 and TCF21 genes, such as rs804280 and rs12190287, were reported to be associated with the risk for congenital heart disease (CHD). Until now the results are contradictory, in this respect we explored whether these two SNPs contribute to the occurrence of CHD.

Materials and Methods: This study consisted of 72 nonsyndromic pediatric patients diagnosed with CHD (atrial septal defect-ASV, ventricular septal defect-VSD and Tetralogy of Fallot-TOF). The control group was represented by 43 healthy subjects. Predesigned TaqMan assays were used for genotyping.

Results: Our results revealed no significant differences of the genotypes between the groups ($p > 0.05$). Also, the alleles distribution of rs12190287 and rs804280 between the groups were approximatively similar, so there were not associated with CHD risk ($OR = 1.54$;95%IC = 0.911–2.68;$p = 0.135$ respectively $OR = 1.25$;95%IC = 0.71–2.21;$p = 0.46$). The distribution of the combined variant genotypes of the investigated SNPs remained approximatively equally between the groups ($p > 0.05$). Even if the CHD group consist in a relatively small number of patients, we investigated the genotypes and alleles distribution separately, analyzing their distribution between controls and ASV patients/VSD patients/TOF patients, but no differences were observed ($p > 0.05$).

Conclusions: The present study revealed that rs12190287 and rs804280 are not associated with CHD risk. While the groups size of our study represents a limitation, we will continue to include new cases and to investigate new SNPs in order to characterize the risk for developing CHD in our pediatric patients.

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E-P05.06
Disease variants associated with coronary heart disease in exomes of Bulgarian centenarians and controls

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Introduction: Cardiovascular diseases cause an estimated annual mortality of 4 million deaths in Europe. Among them, coronary heart disease (CHD) is the most common with overall prevalence of 6.4% in individuals over 20 years. To identify previously reported disease variants linked to CHD that are compatible with longevity and healthy life, we performed WES of the genomes of Bulgarian centenarians and controls.

Materials & Methods: DNA samples from 32 Bulgarian centenarians and 61 healthy subjects (18–30 years) were obtained, two DNA pools were constructed and subsequently whole exome sequenced. We have selected 1351 SNP variants in 705 genes and 190 noncoding regions associated with CHD (https://www.ensembl.org/Homo_sapiens/Phenotype/Locations?oa=EFO:0000378).

Results: We identified 54 variants in centenarians and controls which were previously reported to be associated with CHD. Out of them, 33 variants could not be accepted as predisposing (18 variants have frequency higher than 0,50; 15 have higher frequency in centenarians). Only 5 of the remaining 21 variants have statistically significant (p FDR < 0,05) lower frequency in centenarians compared to controls: *CCDC92* rs11057401 (0,254/0,384); *DHX38* rs1050362 (0,269/0,364); *PDLIM5* rs2452600 (0,297/0,379); *CENPQ* rs2501968 (0,268/0,470); *LIPG* rs2000813 (0,258/0,349). Sixteen variants have statistically insignificant frequency differences between the two pools and could not be considered as having effect on longevity.

Conclusion: Our data demonstrates that an incidental finding of certain reported disease-associated variants may not preclude an extraordinarily long life. The genome of

centenarians can be successfully used to clarify the clinical relevance of genetic variants.

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E-P05.07

Mutation spectrum of the *TNNT2* gene in Russian patients with dilated cardiomyopathy reveals the "hot spot" codon

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Introduction: The prevalence of the hereditary forms of dilated cardiomyopathy (DCM) is 1: 500. Up to 80 DCM-associated genes are known, but most of them do not correspond for >5% of cases. Mutations in the *TNNT2* gene contribute to 3–6% of familial DCM. The aim of this study was to evaluate the spectrum of the *TNNT2* mutations in Russian DCM patients.

Methods: The cohort included 92 probands with DCM, and 36 of them were diagnosed under 18 y.o.. Genetic investigation was performed by NGS of the target genes panel flanking 81 DCM-associated genes including *TNNT2* with following Sanger sequencing of all clinically significant findings.

Results: We revealed three heterozygous pathogenic variants in the *TNNT2* gene in three adult probands with familial DCM, demonstrating both common (heart dilatation) and specific features. The carrier of p.R173Q variant has also left ventricular non-compaction. All substitutions (p.R173Q, p.R173L, and p.R173W) affect the same codon 173, which is placed in the activation domain of troponin T and participates in its complexation with the troponin I.

Conclusion: The mutation rate in the *TNNT2* gene is 3.2% in the whole DCM cohort, and 5.4% in the adult DCM subgroup. This ratio is consistent with the published studies. Mutations affecting arginine 173 were previously reported in familial DCM studies. We suggest that codon 173 might be crucial in myocardial remodeling by dilation and is a mutational "hot spot" in the *TNNT2* gene in Russian DCM patients.

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E-P05.08

A novel pathogenic *TBX5* variant in a family with Holt-Oram syndrome: reverse phenotyping counts

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Introduction: Holt-Oram syndrome (HOS) is an autosomal dominant disorder associated with a broad spectrum of congenital heart defects, arrhythmias, and upper limb malformations. HOS is caused by heterozygous mutations in *TBX5*, a gene essential for cardiac and limb development. Here we report a three-generation family diagnosed with HOS through whole genome sequencing and reverse phenotyping.

Materials and methods: All individuals, two siblings and their mother, had atrium septal defects and later developed sick sinus syndrome and supraventricular arrythmias. In addition, one sibling lost a child and, in a later pregnancy, a foetus due to congenital heart defects. Whole genome sequencing was performed in one sibling. Data analysis was restricted to 203 genes associated with Defect in the atrial septum (HP:0001631) and Sick sinus syndrome (HP:0011704). Sanger sequencing was used for validation and segregation analysis.

Results: A heterozygous, not previously reported, nonsense variant, c.1081>T (p.Gln361*) in *TBX5* (NM_000192) was found to segregate in the family. The mother was shown to be mosaic. Careful reverse phenotyping revealed unilateral mild radial malformation in both siblings. Unfortunately, DNA of the child and foetus were not available for testing.

Conclusion: We report a novel pathogenic variant in *TBX5* that prompted clinical re-examination, leading to a diagnosis of HOS in our family. Our results show that HOS should be considered in patients with seemingly isolated congenital heart defects and/or arrhythmias. Reverse phenotyping is valuable for clinical interpretation of NGS results in cases where the suspicion of an underlying genetic syndrome has not been previously raised.

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E-P05.09

Restricted analysis of multiplex PCR based exome datasets in cardiomyopathy cases

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Introduction: Until recently, more than 40 cardiovascular disorders have been described as a consequence of a single gene defect. Genetic diagnostics is becoming a mainstream practice in the field of cardiology and is recommended in different guidelines. Knowing the underlying genetic defect can help to tailor treatment, define lifestyle recommendations as well as aid in making decisions on placing an ICD or determine the timing of surgical intervention.

Materials and Methods: Total genomic DNA was extracted from the biological sample using a spin column method. We have targeted all of the coding exons with exon-intron boundaries using ultrahigh multiplex PCR-based AmpliSeq exome library preparation method. Sequencing reads were generated on S5 instrument, mapped to the reference genome (hg19) and after variant calling the variants were classified based on ExAc, ClinVar and OMIM information.

Results: The bioinformatic analysis of the datasets were restricted to 326 known cardiological disease causing genes, resulting less variant with uncertain significance and also saved computing time. Our positive cases harboured pathogenic or likely pathogenic alterations in the MYBPC3 (c.1484G>A, c.1776_1777delGT, c.3199_3200insA, c.821 +1G>A), BAG3 (c.1288G>T) and, in the FLNC (c.2713C>T, c.7847A>C) resulting cardiomyopathies with different phenotypes.

Conclusions: Multiplex PCR based library preparation could result highly uniform target coverage with sensitive variant calling. We concluded that if the patients phenotype is certain, in silico gene panel analysis from exome datasets is a faster approach to find the disease causing mutations.

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E-P05.10

Molecular confirmation of Marfan and Loeys Dietz syndrome among Sri Lankan patients with identification of novel likely pathogenic variants

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Introduction: Molecular diagnosis is included in the diagnostic criteria for Marfan syndrome (MFS) and aids Loeys-Dietz syndrome (LDS) diagnosis.

Methods: Review clinical and molecular results of six consecutive tests on suspected MFS cases referred to a clinical geneticist in Sri Lanka.

Results:

Case 1: female (10y) Myxomatous mitral valve prolapse (MVP), myopia, MFS skeletal features. Father and younger sister have similar cardiac, ocular and skeletal findings; paternal uncle and his son clinically MFS. Previously described *FBN1* exon 19 pathogenic variant [c.2243G>A p.(Cys748Tyr)].

Case 2: Female (12y) MFS skeletal features, strabismus, myopia, MVP, large sebaceous naevus of scalp and autism. Insignificantly elevated plasma homocysteine. Previously undescribed, *FBN1* exon 20, likely pathogenic (Class 5) variant [c.2364dupT p.(Val789Cysfs*13)].

Case 3: Male (17y) MFS skeletal features, MVP, normal eye examination. Father died 31y following septicaemia. Unclassified *FBN1* intron 57 c.6998-12T>G class 3 variant predicting extension of exon 58 by 11bp.

Case 4: Female (4y) MFS skeletal features, MVP, divergent squint. *FBN1* exon 26 c.3125G>A p.(Gly1042Asp) variant reported once in MFS and another causal variant with (Glu1042Ser) described.

Case 5: Female (4y) Finger camptodactyly, dilated aortic root, MVP. Previously described *TGFBR2* exon 8 mutation c.1658G>A(p.Arg553His) confirming LDS.

Case 6: Female (10y) Surgery for craniosynostosis and umbilical hernia, MVP, MFS skeletal features. *TGFRBR1* exon 7 c.1198G>A p.(Asp400Asn) pathogenic variant (Class 5) confirming LDS. Variants of uncertain significance of *LTBP3, NOTCH1* and *EMILIN1* were found.

Discussion: Molecular diagnosis of MFS/LDS in Sri Lanka improves management and generates novel information from a previously unstudied population.

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E-P05.11

Detection of novel and reported rare disease-causing genetic mutations in Macedonia: A Collage of Case Reports

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Next generation sequencing approaches have the power to assess hundreds to thousands of genes in parallel and are becoming a first line diagnostic tool in genomic medicine. Herein, we applied clinical exome sequencing or whole exome sequencing in patients presenting with rare cardiovascular disorders. We detected novel and already reported rare and ultra-rare pathogenic mutations in genes known to cause dilatative cardiomyopathy, Long QT syndrome 2, Familial thoracic aortic aneurysm and dissection (TAAD), and dilatative cardiomyopathy caused by Carnitine palmitoyltransferase II deficiency. We validated some of the detected mutations with additional Sanger sequencing or NGS sequencing. To the best of our knowledge, this is the first study to report the role of novel variants such as c.3672C>G, p.Tyr1224* in the *FLNC* gene and c.12964G>A, p.Glu4322Lys in the *SYNE1* gene in dilated cardiomyopathy, and c.1850A>G, p.His617Arg in the *CPT2* gene in cardiomyopathy caused by CPT II deficiency. Besides that, we report additional known rare and ultra-rare VUS that according to our study represent pathogenic/potentially pathogenic variants involved in cardiovascular diseases.

Keywords: next-generation sequencing, rare congenital cardiovascular diseases, cardiomyopathy, long QT syndrome, TAAD

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Novel and known rare mutations identified in this study

Patient	NGS test	Diagnosis	Gene	c.DNA	protein	rsID	MAF (GnomAD/ ExAC/1000G)	Clinvar	ACMG	Novelty	Inheritance
P1	WES	Dilatative cardiomyopathy	*FLNC*	c.3672C>G	p.Tyr1224*	/	/	/	PVS1, PM2, PP3	Unknown	AD
P1	WES	Dilatative cardiomyopathy	*MYBPC3*	c.1783A>G	p.Ile595Val	rs730880550	/	/	PM1, PM2, PP2	Known VUS, extremely rare	AD
P1	WES	Dilatative cardiomyopathy	*MIB1*	c.1552C>T	p.Arg518*	rs769209279	2,845E-05	/	PP3	Known VUS, extremely rare	AD
P1	WES	Dilatative cardiomyopathy	*KCNQ1*	c.1349A>G	p. Glu450Gly	rs1057518902	/	/	PM1, PM2, PP2	Known VUS, extremely rare	AD
P2	CES	Congenital arrhythmia	*KCNJ2*	c.566G>T	p.Arg189Ile	rs199473381	/	Likely pathogenic	PM2, PM5, PP2, PP3, PP5	Known VUS, extremely rare	AD
P3	CES	Familial TAAD	*TGFBR3*	c.2423A>G	p. Tyr808Cys	rs746368140	3,251E-05	/	PP3	Known VUS, extremely rare	AD
P4	Gene panel	Cardiomyopathy	*VCL*	c.1352 +5G>A	/	rs374522164	4,47E-05	Uncertain significance	PP3	Known VUS, extremely rare	AD
P5	WES	Long QT syndrome 2	*KCNH2*	c.2948C>T	p.Thr983Ile	rs149955375	0.000152	Uncertain significance	PM1, PP3, PP5	Known VUS, extremely rare	AD
P5	WES	Dilatative cardiomyopathy	*SYNE1*	c.12964G>A	p. Glu4322Lys	/	/	/	PM2	Unknown	/
P5	WES	Dilatative cardiomyopathy	*MYPN*	c.3833G>A	p. Arg1278Gln	rs142877365	0.000451	Uncertain significance	/	Known VUS, extremely rare	AD
P6	WES	Dilatative cardiomyopathy; CPT2 deficiency	*CPT2*	c.338C>T	p. Ser113Leu	rs74315294	0.00138	Pathogenic	PP3, PP5	Known, rare	AR
P6	WES	Dilatative cardiomyopathy; CPT2 deficiency	*CPT2*	c.1850A>G	p. His617Arg	/	/	/	PM2	Unknown	AR

E-P06
Metabolic and mitochondrial disorders

E-P06.01
Association of ABCB4 Gene Polymorphism with Lipid metabolism in Overweight Children

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Introduction: A major concern is the excess nutrient intakes in children as a driver to obesity. Obesity is associated with dyslipidemia and type-2 diabetes. Genetics seems to play an important role in obesity, as various gene polymorphisms may influence lipid metabolism. The ABCB4 gene encodes a protein involved in the transport of phospholipids from hepatocytes into the bile.

Objective: The aim was to evaluate the association of several *ABCB4* polymorphisms with plasma levels of cholesterol, triglycerides and PUFAs in overweight children.

Methods: The cohort consisted of 200 overweight children, aged 7–18, (97 males, 103 females) with BMI > +2 SD as compared to the WHO reference, and abdominal circumference above the 90th percentile. Total cholesterol, HDL-c, triglycerides and PUFAs were measured in plasma samples. LDL-c levels were calculated using Friedewald equation. Next generation sequencing was used for identification of 6 ABCB4 gene polymorphisms (rs1149222, rs2071645, rs31672, rs4148811, rs9655950, and rs1202283).

Results: By comparing the levels of cholesterol (Total-c and LDL-c), triglycerides and PUFAs, we found significant differences in the plasma levels for linoleic acid, Total-c and LDL-c for the groups identified based on one ABCB4 genotype. Children with rs2071645 variant had lower levels of Total-c, LDL-c and linoleic acid as compared to children having the wild type variant.

Conclusion: The results indicated that the ABCB4 rs2071645 polymorphism may have a protective effect against dyslipidemia in overweight children.

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E-P06.02
A case with a homozygous novel mutation in *AGPS* with non-rhizomelic body structure

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Introduction: Alkylglycerone Phosphate Synthase (*AGPS*) gene encodes a protein that localized to the inner aspect of the peroxisomal membrane and plays role at plasmalogen synthesis. Rhizomelic chondrodysplasia punctata (RDCP) is a rare autosomal recessive disorder with shortening of proximal extremities, punctate calcification of cartilage, flexion contractures, vertebral clefts, congenital cataracts, seizures, severe growth deficiency and intellectual disability. We report a non-rhizomelic case with a novel mutation in *AGPS* which is responsible for RDCP type 3.

Materials and Methods: After performing targeted next-generation sequencing including *FAR1* gene, Illumina TruSight Inherited Disease panel including *PEX7, PEX5, AGPS* genes was performed.

Results: A 4-year-old male who had a history of operated congenital cataract, flexion conractures, seizures, growth deficiency and intellectual disability was consulted to the genetics department with clinical suspicion of Peroxisomal Fatty Acyl-CoA Reductase 1 disorder and RDCP. The case had growth retardation and microcephaly. Extremities weren't rhizomelic although X-rays showed stippled epiphyses. *FAR1* analysis was normal. Inherited Disease panel revealed a novel homozygous variant at *AGPS*, c.1475+3 A>G, in the patient. Parents who have consanguinity were heterozygous for the same variation. It is predicted to be disease causing by disrupting the splice region of the exon 14.

Conclusion: While all RCDP genotypes are associated most commonly with the classical phenotype, milder phenotypes like having non-rhizomelic limbs have been described. Our case is noteworthy in terms of both having a new mutation at *AGPS* and being non-rhizomelic.

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E-P06.03

First genetically proven Arginase deficiency case of Bulgarian Roma origin due to a novel pathogenic splice site variant in the ARG1 gene

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Argininemia [MIM #207800] is a rare metabolic disease which is characterized by a defect in the final step of the urea cycle. The gene *ARG1* encodes arginase which catalyzes the hydrolysis of arginine to urea and ornithine. Pathogenic variants in *ARG1* gene can cause Arginase deficiency. Here we report a 14 years old Gypsy boy from Bulgaria. The patient has respiratory distress syndrome in early neonatal period. A regress in his development at age of 9 months was observed. At 1 year and 8 months he was diagnosed with epilepsy. Blood ammonia was slightly elevated—102,9 μmol/l (normal range 10–47 μmol/l). Based on the clinical symptoms, Sanger sequencing of the *ARG1* gene was performed. The molecular-genetic analysis showed a novel homozygous donor splice site variant in the *ARG1* gene: c.329+1G>A. Segregation analysis in the family showed the same variant in heterozygous state in both parents. The detected variant was not present among 138.000 controls of the gnomAD project. The result from the tool for theoretical pathogenicity prediction (Human Splicing Finder) supports possible splicing effect, which is pathogenic. In conclusion, we present the first genetically verified patient with Arginase deficiency in Bulgaria. We established a novel pathogenic variant in the *ARG1* gene in a patient from Roma community, born to consanguineous parents. We hypothesize a possible private variant for the Gypsy population in Bulgaria. The study was supported by Medical University Sofia, Contract number D-116/2018.

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E-P06.04

Family study of the first case of autosomal recessive hypercholesterolemia in the Czech Republic caused by a new variant in the LDL receptor adaptor protein 1 gene

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Introduction: Autosomal recessive hypercholesterolemia (ARH; OMIM catalog number # 6038133) is a rare, inherited lipid metabolism disorder. This disorder is caused by loss of adaptor protein function, encoded by LDL receptor adaptor protein 1 gene (*LDLRAP1*).

Materials and Methods: Next generation sequencing of the *LDLR*, *PCSK9* and *APOE* genes and part of *APOB* gene (exon 26) using ADH MASTR kit (Multiplicom, Belgium); Sanger sequencing of the *ABCG5*, *ABCG8* and *LDLRAP1* genes. DNA samples have been collected within the framework of the MedPed project.

Results: Performed sequence analysis of the *LDLRAP1* gene revealed a new variant in the 2nd exon, c.143T>C; p.(Phe48Ser) in a homozygous constitution in the proband and in a heterozygous state in all 4 her brothers. This sequence variant is located in the phosphotyrosine-binding domain, which is responsible for binding to the NPXY internalization signal of the LDL receptor protein. ACMG criteria were used for pathogenicity evaluation of founded sequence variant. There was relatively good lipid-lowering drugs therapy response in our ARH proband. This finding is in agreement with the previously published studies.

Conclusions: We descripted the first case of Czech family with a rare mutation in the LDR receptor adaptor protein 1 gene, which cause autosomal recessive hypercholesterolemia. There are only few cases of ARH mutations described worldwide.

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E-P06.08

Are *COX11* variants responsible for autosomal recessive Mitochondrial Complex IV Deficiency?

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Introduction: Mitochondriopathies are a clinically heterogeneous group of disorders that arise from the variations of genes encoded by either mitochondrial DNA (mtDNA) or nuclear DNA. Common clinical features include external ophthalmoplegia, proximal myopathy, cardiomyopathy, sensorineural deafness, optic atrophy, diabetes mellitus and abnormal plasma/CSF lactate/pyruvate levels.

Materials and Methods: Whole exome sequencing (WES) analysis performed in a 2.5-month-old preterm girl with persistent feeding intolerance and high lactate, and her parents. WES analysis did not yield a diagnostic result. Therefore, a scientific approach focusing on disease candidate genes are performed due to the informed consent of the family.

Results: WES revealed a homozygous frameshift variant in *COX11* (OMIM *603648), resulting in a premature stop codon, and subsequent mRNA degradation (nonsense-mediated decay) or truncation of the protein [c.35_36delinsG, [p.(Val12Glyfs*21)]. Both parents are heterozygous carriers.

Conclusion: COX11 is a nuclear-encoded subunit of cytochrome c oxidase or Complex IV (COX; EC 1.9.3.1), comprised of 13 polypeptide subunits, 3 of which are encoded by mtDNA (*MT-CO1, MT-CO2, MT-CO3*). Several nuclear-encoded genes directly encoding the subunits or maintaining their activities are known for Mitochondrial Complex IV Deficiency (OMIM #220110) which is clinically heterogeneous, ranging from isolated myopathy to severe multisystem disease. In addition to clinical medical analysis, reporting scientific findings in disease candidate genes is a crucial task of the diagnostic laboratory settings in the era of Next Generation Sequencing for expanding the knowledge base. In order to be able to report such scientific findings, informed consent of the families will be required.

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E-P06.09
Fabry disease - diagnostic challenges in children

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Fabry disease, is an X-linked metabolic disorder manifested in males with angiokeratoma, acroparestesia, cardiomyopathy, nephropathy and early stroke. Clinical features develop with age, the diagnosis being challenging in children. Females have a very variable clinical picture due to X chromosome inactivation. We shall present 4 boys diagnosed with Fabry disease in Iasi Regional Medical Genetics Centre to present diagnostic and management challenges we have encountered. All cases were confirmed with enzymatic and DNA test. Case 1: 26 years old, presented at 15 years with angiokeratoma and acroparestesia. Echocardiography revealed mild cardiomyopathy. Enzyme replacement therapy (ERT) started the progression of the disorder. However, at 22 years he developed sudden deafness, corrected by ENT specialists. Family history is highly suggestive. Case 2: 14 years old, came to medical attention at 10 years due to short stature and anemia. Physical examination revealed a few angiokeratoma and investigations showed chronic renal failure and marked cardiomyopathy. ERT corrected cardiomyopathy, but he had to follow renal transplant. Family history is positive, but not suggestive.clinical picture in time Case 3: 21 years old, diagnosed at 18 years after developing rapidly progressive renal failure. No other features were present, but storage was proven in the renal biopsy. Family history is positive, but not suggestive. Case 4: 17 years old, admitted to hospital for a different reason. Physical examination revealed angiokeratoma and acroparestesia. Family history is positive, but not suggestive. In conclusion, we present 4 children with Fabry disease to underline early features, evolution in time and particularities.

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E-P06.11
Genetic characteristics of patients with glutaric aciduria type I from Ukraine

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Introduction: Glutaric aciduria type I (GA-I) (MIM 608801) is an autosomal recessive inherited disease caused by rearrangements in *GCDH* gene, which encode glutaryl-CoA dehydrogenase. Nowadays, 244 mutations are described in *GCDH* gene according to HGMD. The most common mutations are p.R402W in patients from Europe and p.A421V in population of Amish in the USA. In

Ukraine spectrum of mutations of patients with GA-I haven't been studied yet.

Materials and methods: dry blood spots and venous blood with EDTA of 5 patients (aged from 7 month to 8 years old); DNA-sequencing by Sanger.

Results: In the study 100% of mutant alleles in *GCDH* gene in patients with GA-I from Ukraine were detected. Spectrum of pathogenic variants was represented by 2 common mutations: p.R402W (3/10 alleles) and p.A421V (3/10 alleles); 2 rare mutations p.R383C (2/10 alleles) and p.G390R (1/10 alleles), all mutations were identified in heterozygous state despite the rare variant p.R383C (1 homozygote). Also, we found one novel mutation p.D396G (1/10 alleles). Prognostic analysis showed that this novel variant p.D396G is pathogenic (Polyphen2—0,853, Provean—(−6,394)). All detected mutations were localized in 10, 11, 12 exons of *GCDH* gene. Analysis of GA-I patients' parents origin showed that 50% of pathogenic alleles were from Western part of Ukraine.

Conclusion: Missense variants p.R402W and p.A421V are most common within Ukrainian patients with GA-I. In order to confirm diagnosis GA-I for Ukrainian patients it is expedient to primarily examine 10, 11, 12 exons of *GCDH* gene.

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E-P06.12
Variable clinical and genetic manifestations of Wilson disease in two North-African families

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Introduction: Wilson disease (WD) is a disorder caused in most cases by biallelic pathogenic variants in *ATP7B* gene, resulting in abnormal copper metabolism leading to a varying combination of hepatic, neurologic, and psychiatric features. Diagnosis is established on clinical and biochemical features, and confirmed by mutation analysis of *ATP7B* gene. We report on the clinical and molecular findings in three cases from two North-African families with WD.

Materials and methods: The first case, a 20-years-old Tunisian female, was born to a consanguineous union. She had two sisters and a brother who died in early childhood from hepatic manifestations of WD. The patient presented with recurrent jaundice, neurologic and psychiatric manifestations. Biochemical tests showed low ceruloplasmin concentration and high cupruria.

The second and third cases were two male brothers born to non-consanguineous Algerian parents. The elder patient, presented with an hepatic form of WD associated to kayser-Fleisher ring, decreased ceruloplasmin, and increased cupruria. Biochemical screening in the patient's sibs found decreased ceruloplasmin and high cupruria in a 8-year-old asymptomatic brother. A sequencing of the whole coding regions of *ATP7B* gene was performed in the three cases.

Results: No pathogenic variant was found in *ATP7B* gene in case 1. Two variants in the compound heterozygous state were found in the second and third cases. Each one of the healthy parents was a heterozygous carrier of one variant.

Conclusions: Sequencing of *ATP7B* gene is very important when WD is suspected as it allows to give an appropriate genetic counseling.

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E-P06.14
Whole mitochondrial genome analysis in carriers of mt3460 mutation with Leber's hereditary optic neuropathy

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Background: Leber's hereditary optic neuropathy considered one of the most frequent neurodegenerative mitochondrial diseases in which the mutated or impaired mitochondria implicated in retinal ganglion cell (RGC) loss and optic nerve degeneration. So LHON clinically manifested by blurred vision, followed by gradual painless loss of vision in both eyes within a short time that eventually leads to devastating loss of central vision.

Objective: This study has been provided a mutational screening for full mt-DNA by Sanger sequencing in Serbian

patients carriers of mt3460 G>A mutation for detection of secondary changes associated with LHON.

Material and Methods: Individuals included in this study were recruited from the Child and Adolescent Neurology and Psychiatry Clinic and from Neurology Clinic CCS, Belgrade, Serbia. All examined individuals had characteristic clinical presentation suggesting the presence of LHON. Multiple segmental PCR amplicons of mtDNA have been sequenced by Sanger's method and the obtained results were compared with the Revised Cambridge Reference Sequence "rCRS ".

Results: We have detected mt.3460 G>A mutation in ND1, in 3/19 LHON families. In 3460G>A positive LHON cases, additional mtDNA mutations that co-occur with primary mutation are identified as secondary/intermediate LHON mutations: T4216C, G13708A, G15257A, and G15812A, in addition to multiple polymorphic variants (C295T, T489C, A11251G, A12612G C16069T, and T16126C). Our finding is the first instance showing that mt3460 G>A mutation in Serbian LHON subjects is associated with variants belonging to mitochondrial haplogroup J.

Conclusion: Wide spread European mitochondrial haplogroup J may modulate the phenotypic manifestation of LHON in carriers of mt3460G>A mutation.

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E-P06.15

A novel frameshift mutation in the melanocortin-4 receptor gene (*MC4R*) in two unrelated families with severe early-onset obesity

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Introduction: The melanocortin-4-receptor (MC4R), expressed in the central nervous system, is a part of the leptin-melanocortin signaling pathway and an important regulator of appetite and energy homeostasis. *MC4R* mutations are the most common cause of monogenic obesity, accounting for approximately 2–5% of severe childhood obesity with variable penetrance.

Materials and methods: We used a custom-made targeted exome sequencing panel to identify genetic variants associated with early-onset obesity in subjects with severe obesity (height-adjusted weight >60%) before age 7 years.

Results: Targeted exome sequencing identified a novel heterozygous frameshift deletion that introduces a premature termination of translation NM_005912 (MC4R): c.308delT, (p.Val103fs), in two index cases, an 18-year-old male and a 20-year-old female. The overweight mother of the female index carried the same mutation. The deletion leads to a truncated protein and likely results in loss of receptor function. The two index patients had persistent obesity since childhood and the male index had BMI 35 (BMI Z-score 3.0) and the female index had BMI 44 (BMI Z-score 4.3), hyperphagia and type 2 diabetes.

Conclusion: Our findings support that mutations in the melanocortin pathway are related to severe obesity. Screening for monogenic obesity in patients with early-onset obesity and hyperphagia should be considered as it enables early intervention, potential treatment and helps to reduce stigma of obesity. MC4R is a promising target for development of anti-obesity therapy.

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E-P06.16

Genetic characterisation of a Maltese cohort with atypical non-autoimmune diabetes

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Introduction: Maturity onset diabetes of the young (MODY) is a heterogeneous group of early-onset non type 1 diabetes caused by single gene defects in a number of genes affecting pancreas development and beta-cell function. Obtaining an etiological molecular diagnosis in suspected monogenic diabetes is important for both a correct clinical management and genetic counselling of patients at-risk. Genetic testing for MODY is a challenging process, in view of the clinical heterogeneity of cases and the possible overlap in phenotypes with both type 1 and type 2 diabetes. Furthermore, the large number of genes implicated in MODY makes screening for mutations by Sanger sequencing an expensive and time-consuming process.

Methods: 31 adult patients with suspected monogenic diabetes and a high probability of MODY were recruited.

Whole exome capture using a SeqCap-EZ-MedExome kit, followed by paired end sequencing was performed on each sample, in collaboration with the European Genomic Institute for Diabetes. We carried out gene-focused analysis of all known MODY genes to filter and prioritize pathogenic variants.

Results: We present the clinical characteristics of the probands and the relevant genetic findings from the cohort are discussed. A number of rare mutations in several genes were detected, and we present evidence for possible genetic founder effects.

Conclusion: Our findings are novel to a regional population, where the genetic epidemiology of MODY has never been characterised. The use of high-throughput sequencing facilitates the detection of both known mutations in a defined gene panel and novel unreported rare variants as possible diabetes-causing mutations.

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E-P06.18
Analysis of genes associated with obesity phenotypes in mouse models, reveals new potentially causative mutations for monogenic obesity in humans

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The discovery of genes responsible for highly penetrant forms of monogenic obesity and type II diabetes has been underpinned by knowledge gained from analysis of mouse models. Exome sequencing of obese humans, particularly with extreme phenotypes, has since resulted in a steady stream of new causative genes being identified, and we postulate that more are yet to be discovered. Mouse phenome databases represent a rich source of unexplored candidate genes for potentially causative of human monogenic obesity. Here we present the results of re-analysis of whole exome sequencing from 70 people with severe obesity (BMI>50), from a cohort of individuals seeking weight loss/metabolic surgery in the UK. The data were examined specifically for rare, predicted deleterious mutations in genes that, when mutated, result in obesity phenotypes in murine models. In this pilot dataset, we found a number of rare missense and insertion/deletion mutations in PEG3, PIK3C2G,ATXN2 and HRH3 genes (in both heterozygous and homozygous condition). In addition to the phenotypic data from mice, SNPs in ATXN2 and PIK3C2G have been reported to be associated with human BMI and related phenotypes while PEG3 expression has also repeatedly been reported to be important in pancreatic beta cell renewal and function: notably 7/8 PEG3 mutation carriers had concommittent type II diabetes. Thus, our results add to the accumulating list of genes putatively responsible for highly penetrant forms of human obesity and/or diabetes.

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E-P06.20
Correlation of Vitamin D deficiency and PKU genotype in Georgian patients

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Introduction: Patients with PKU can develop deficiency of several vitamins. Aim of the study was to find correlation between the genotype and Vitamin D deficiency level in 148 Georgian PKU patients.

Materials and methods: Study was made in Georgian population and the results of 148 patients were analyzed. 25 (OH) D Vitamin was measured in the blood plasma of PKU patients. The only Vitamin D intake during last 3 month was exclusively from amino acid formula (8.7 mg in 100 g).

Results: In 20 patients with PAH mutation P281L on both alleles, the mean of Vitamin D was 12,8 ng/ml, in 61 patients with mutation P281L on one allele, the mean of Vitamin D was 17.6 ng/ml, in 61 patients with mutation other than P281L on both alleles the mean of Vitamin D was 22.1, and finally, in 7 patients with HPA Vitamin D mean made 35.3 ng/ml. Based on our study, only 8,6% of investigated 148 patients showed normal rate of Vitamin D (30.1–100 ng/ml) in the blood.

Conclusions: There is a noticeable correlation between the genotype of PKU patients and the concentration of Vitamin D in blood. PKU patients with homozygous mutation P281L are more affected with low concentration of Vitamin D, then the patients with the same mutation on one allele, and the PKU patients with all the other mutations

are less affected with Vitamin D deficiency. There is a need to supplement patients with the Vitamin D through taking in consideration their genotype.

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E-P07
Immunology and hematopoietic system

E-P07.01
How we treat CML patients in developing countries: 13 years experience

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Introduction: TKI therapy for CML patients, including imatinib and nilotinib, was gradually introduced in Bosnia and Herzegovina because of financial considerations and lack of insurance cover, which had drastic consequences on patients' outcomes. Aim of this study was to compare the long-term clinical outcomes of CML patients receiving delayed and immediate front-line TKI therapies.

Methods: Newly diagnosed CML-CP patients (n = 178) treated with TKI in period from August 2005 to December 2018 were included in this multicentre retrospective cohort study. Patients were on front-line imatinib (Group 1, n = 135) and on front-line nilotinib (Group 2, n = 43). Further subcategories were made based on the duration of treatment delay: <5 months, 6–13 months and >13 months for imatinib treatment and <6 months and >6 months for nilotinib treatment. Survival probabilities were estimated with the Kaplan-Meier method using the log-rank test.

Results: Median follow-up was 43 months and 58 months, respectively. OS at 13 years in Group 1 and Group 2 was 84.6 and 90.7%, respectively. CCyR and MMR at 24 months were higher in Group 2 compared to Group 1 (90 *vs* 75% and 79 *vs* 64%, respectively). For delayed imatinib treatment (<5 months, 6–13 months, >13 months) at 24 months, CCyR was 75 *vs* 64 *vs* 40%, respectively. Regarding nilotinib treatment (<6 months, >6 months), patients achieved 93 *vs* 82% for CCyR and 82 *vs* 77% for MMR, respectively.

Conclusion: Front-line nilotinib showed improved efficacy over front-line imatinib. No significant differences were found among subgroups of patients on front-line nilotinib.

E. Islamagic: None. **S. Kurtovic:** None. **A. Hasic:** None. **A. Kurtovic-Kozaric:** None.

E-P07.02
When we must suspect a genetic cause of hyper IgE syndrome ?

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Introduction: Hyper IgE syndrome is usually associated with allergic phenomenas such as asthma, dermatitis, parasitosis. But hyper-IgE in a patient presenting eczema, asthma associated with recurrent skin and respiratory tract infections, should raise the question of an underlying immunodeficiency.

Case presentation: A 11 year old boy with a long history of multiple skin and respiratory tract infections of bacterial, viral and fungal etiology and asthma that started in early childhood is addressed to our hospital. Clinical examination revealed eczema, hand warts, hypo- and hyperpigmented facial lesions, severe oral candidiasis, inspiratory dyspnea, productive cough, and severe dysphonia. Laboratory investigations showed lymphopenia, eosinophilia (1500/mm^3), very elevated IgE values (>2500 IU/ml) and hypogammaglobulinemia (Ig G 5,54 g/l). The indirect videolaringoscopy showed juvenile laryngeal papillomatosis. All these findings suggested a primary immunodeficiency. The clinical and laboratory aspects of Job's syndrome and *DOCK8* deficiency are overlapping but distinct. The absence of connective tissue and skeletal abnormalities (scoliosis, hyperextensibility, pathologic fractures, retained primary dentition) helped distinguish between these two rare syndromes. The latter has been confirmed by absence of DOCK8 protein. Management of DOCK8 deficiency includes prophylactic antibiotic, antifungal, and antiviral therapy. In view of the high risk of early death from opportunistic infection or malignancy, stem cell transplantation is considered to be the curative treatment.

Conclusions: Any hyper-IgE syndrome in children presenting recurrent respiratory and viral skin infections should raise the suspicion of a genetic immunodeficiency. The diagnosis must obtain earlier as possible, bone marrow transplantation being the unic curative treatment.

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E-P07.03

A single center experience in Turkey in the molecular diagnosis of Hemophilia B

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Hemophilia B (HB) is an X linked recessive bleeding disorder with a prevalence of 1 in 30,000 live male births. Bleeding symptoms vary according to the level of coagulation factor IX activity. To date more than 1200 mutations have been defined in *F9* gene. The aim of this study was to determine the mutation spectrum of *F9* gene in HB patients from Turkey and to establish a phenotype-genotype correlation.

Twenty eight HB patients, who were molecularly analyzed for *F9* gene mutations in Ege University Pediatric Genetics Laboratory, were included in the study. Clinical and laboratory findings were obtained from hospital records. The Factor IX Gene Variant Database and Human Gene Mutation Database were searched for the identified mutations. Pathogenicity of the variants was classified in accordance with ACMG criteria.

From the 28 HB patients 12 (42.9%) were classified as severe, 13 (46.4%) moderate and 3 (10.7%) mild. Missense, nonsense and splice site mutations were identified in 16 (57.1%), 8 (28.6%) and 4 (14.3%) of the study group, respectively. Five mutations (c. 89-2_89-1insT, c.521-1G>A, c. 839-1G>T, c. 1088G>T, and c. 1238G>T) were novel. Two of the 5 patients with novel mutations had moderate phenotype while the remaining 3 had severe phenotype.

In conclusion, in our study group, mutations in F9 gene could be found anywhere throughout the entire gene; as in previous studies. This study may contribute to the phenotype-genotype correlation of HB due to the 5 novel mutations mentioned herein.

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E-P07.04

Implication of HLA-G +3142G/C polymorphism and soluble HLA-G in mastectomized breast cancer patients in Tanzania

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Introduction: HLA-G is an immunosuppressive molecule existing in a soluble (sHLA-G) and membrane-bound form. It's thought to be expressed more by tumor cells and hence contributing to immune evasion. The HLA-G +3142G/C polymorphism (rs1063320) post-transcriptionally affects the expression of HLA-G. This molecule and SNP have been implicated in breast cancer (BC) and others, but with limited studies in African population.

Aim: The aim was to determine whether sHLA-G and rs1063320 are relevant in BC in Tanzanian population.

Materials and Methods: The study consisted of 75 BC patients and 84 normal controls. 81.3% had undergone mastectomy. Plasma sHLA-G was quantified by ELISA. Genotyping was done by LightSNiP typing assay using quantitative Real-Time PCR.

Results: The sHLA-G level was significantly lower in patients and mastectomized patients as compared to controls ($p < 0.01$) and non-mastectomized patients($p = 0.018$) respectively. sHLA-G was not relevant to metastatic and receptors expression (ER, PR and HER2) status. The frequencies distribution of rs1063320 genotypes and alleles were relatively similar between patients and controls. There was no significant influence of rs1063320 genotype on sHLA-G level. The Kaplan-Meir analysis revealed no significant difference between patients carrying at least one risk allele versus no risk allele(GG) in their likelihood to have metastatic free survival (Log rank, $p = 0.6508$).

Conclusion: While changes in sHLA-G levels in response to medical interventions such as mastectomy may be translated into its potential use as a prognostic

marker for BC, the rs1063320 may not solely affect the sHLA-G level and reliably serve as a genetic risk factor for BC.

I.C. Adolf: None. **G. Akan:** None. **N. Dharsee:** None. **T. F. Mselle:** None. **F. Atalar:** None.

E-P07.05
Clinicopathological characteristics of patients with myeloid neoplasms from Bosnia and Herzegovina: 20 year follow-up

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Introduction: Treatment of patients with myeloid malignancies is suboptimal in Bosnia and Herzegovina due to the lack of targeted therapies. The aim of this study was to collect all available data in the last 20 years.

Methods: Patients with myeloid neoplasms (n = 271) treated at the Clinical Center of the University of Sarajevo, B&H, were included in this study (MPN n = 106, AML n = 64, MDS n = 52, CML = 47). Patient data was collected including age at diagnosis, sex, blood parameters, molecular-cytogenetic findings, therapy, and survival.

Results: *JAK2*^{V617F} was detected in 41% of MPN patients, 6.60% had a *CALR* gene mutation, *MPL* mutation had 2%, while 5% reported abnormal cytogenetic findings. 35.8% of MPN patients had a fatal outcome, while 47.20% had survival ≥ 60 months. CML patients enrolled in this study received imatinib, nilotinib (60%, n = 28), or never received TKI (40%, n = 19). Patients on nilotinib achieved CCyR faster than patients on imatinib. 28% (n = 13) of CML patients had a fatal outcome. Abnormal molecular-cytogenetics of MDS patients was found in: 12.24% had (7q)/- 7, del(5q) and +8 had 8.16% respectively, and a complex karyotype had 6.12%. According to IPSS-R score, the majority had median (12.24%) and high (10.2%) risk. A total of 63.27% MDS patients died, and five-year survival was 24.48%. Molecular-cytogenetic abnormalities of AML patients were found in 55%. 52% of AML patients achieved remission, lasting on average 19 months, while 22% patients had survival ≥24 months.

Conclusion: Clinical outcomes of MN patients were suboptimal.

A. Kurtovic-Kozaric: None. **L. Mehinovic:** None. **E. Islamagic:** None. **A. Dizdarevic-Rekic:** None. **H. Komic:** None. **S. Kurtovic:** None.

E-P07.06
Clinical outcomes of patients with *JAK2*, *CALR* and *MPL* positive myeloproliferative neoplasms

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Introduction: Myeloproliferative neoplasms are a group of clonal myeloid disorders. The aim of this study was to evaluate disease phenotypes and evolution in *JAK2, CALR* and *MPL* mutations in the population of MPN patients in Bosnia and Herzegovina.

Materials and methods: We included 138 MPN patients (PV (n = 41), ET (n = 56), PMF (n = 10) and MPN-U (n = 31)). Three genes were analysed: *JAK2*, *CALR*, and *MPL*. *JAK2*^{V617F} allele burden was performed by qPCR (Ipsogen MutaQuant, Qiagen). Statistical analysis was conducted using SPSS v21.

Results: Table 1 reports clinical parameters and mutational status of MPN patients. *JAK2*^{V617F} was found in 71%, *CALR* (type 1 and 2) in 13%, and *MPL* (W515L and W515K) in 4% of MPN patients. *JAK2*^{V617F} PV patients had higher platelet count. *JAK2*^{V617F} ET patients had higher RBC levels, and lower platelets compared to *CALR*+ ET patients. *JAK2*^{V617F} ET versus PV patients showed lower values for RBC, Hb and Hct, higher values for platelets, and higher frequency of splenomegaly. When we compared *JAK2*^{V617F} PMF, ET, and PV patients, PV patients had higher RBC, Hb, and Hct values. The *JAK2*^{V617F} allele burden was directly correlated with Hb, RBC, and Hct and inversely correlated with platelet count. MPN-U patients had the worst survival among MPN subgroups.

Conclusion: Mutational signatures affect the phenotypic presentation of MPN subtypes. Mutant allele burden is a determinant of the phenotypic features of *JAK2*^{V617F} MPN.

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	PV		ET					PMF			
	JAK2+ (N = 25)	NA (N = 9)	*JAK2*+ (N = 31)	*CALR*+ (N = 8)	*MPL*+ (N = 2)	TRIPLE NEGATIVE (N = 4)	*NA* (N = 11)	*JAK2*+ (N = 6)	*CALR*+ (N = 2)	*MPL*+ (N = 1)	TRIPLE NEGATIVE (N = 1)
Male/ Female (% Male)	9/16 (36%)	8/1 (89%)	9/22 (29%)	5/3 (63%)	1/1 (50%)	0/4 (0%)	4/7 (36%)	4/2 (67%)	1/1 (50%)	0/ 1 (100%)	1/0 (100%)
Age, Y	70 (29–84)	69 (61–81)	66 (40–82)	68 (37–84)	62 (56–68)	67.5 (28–87)	69 (39–78)	63.5 (25–78)	56 (47–65)	56	32
Rbc	6.67 (2.87–8.32)	5.82 (4.60–7.77)	5.01 (3.22–7.29)	4.85 (3.35–5.03)	4.5 (4.29–4.71)	4.51 (4.41–5.14)	4.73 (2.68–7.94)	4.88 (3.57–5.63)	4	4.46	1.89
Hemoglobin (Hb)	169 (118–217)	173 (155–207)	146 (78–169)	143.5 (118–156)	130.5 (129–132)	144.5 (134–151)	147 (118–194)	138 (89–149)	121.5 (118–125)	115	35
Hematocrit (Hct)	0.55 (0.35–0.66)	0.53 (0.47–0.63)	0.44 (0.25–0.55)	0.43 (0.36–0.45)	0.38	0.43 (0.39–0.45)	0.44 (0.33–0.59)	0.41 (0.31–0.47)	0.34	0.29	/
MCV	82 (56.8–124)	87 (80.6–101)	87.5 (73.4–102)	89.5 (85–95.6)	89	92.05 (88.2–95.2)	88 (74–114)	80.8 (78–103)	86	77	/
WBC	10 (5.2–16.9)	7.10 (5.13–13.00)	11.15 (6.06–15.97)	9.98 (6.5–18.01)	11.09 (10.98–11.2)	9.63 (5.1–12.5)	9.35 (3.3–19.4)	18.55 (1.63–40.8)	8.16	9.85	5.5
Neutrophils	68.5 (41.7–82)	59.11 (42–80.2)	68.50 (43.2–75.6)	60.8 (46–85.6)	65.00	66.68 (61.45–71.9)	71.93 (53.9–90.9)	75.9 (62.6–84)	/	69.64	/
Eosinophils	2 (0.3–6.15)	1.85 (1–2.93)	1.60 (0.129–5.3)	1.49 (1–1.98)	1.80	2.25	2.38 (0.161–4.68)	2.63 (2–3.43)	2.14	1.42	/
Basophils	0.8 (0.1–3.01)	1 (0.83–1)	1.03 (0.263–3.57)	0.34 (0.04–0.64)	0.91	1.49 (1.06–1.92)	0.68 (0.102–1.25)	1.8 (1.18–3)	/	1.3	/
Platelets (Plt)	471 (97.3–1650)	266 (151–532)	834 (367–1438)	1155.5 (848–1250)	977 (922–1032)	648 (448–1051)	927 (530–1643)	342 (158–2250)	284.5 (157–412)	1479	140
Splenomegaly	5/14 (36%)	3/3 (100%)	3/14 (21%)	1/6 (17%)	1/2 (50%)	1/3 (30%)	1/9 (11%)	4/4 (100%)	1/1 (100%)	1/ 1 (100%)	/
Hepatomegaly	1/10 (10%)	0/4 (0%)	2/12 (17%)	0/5 (0%)	1/2 (50%)	0/2 (0%)	1/8 (13%)	2/3 (67%)	/	1/ 1 (100%)	1/1 (100%)
Ldh	381.5 (217–1876)	321 (159–721)	282 (167–521)	345 (183–553)	261.5 (175–348)	387 (161–613)	337 (235–427)	609 (254–1697)	1166.5 (1102–1231)	410	/
Bilirubin	12.7 (7.6–19.5)	33.1	10	/	23.1 (7.4–38.8)	/	7.2	31.95 (22–41.9)	/	/	/
Therapy	Hydroxiurea, Litalir, Allopurinol, Controloc, Aspirin Protect	Hydroxiurea, Litalir, Allopurinol, Controloc, Aspirin Protect	Hydroxiurea, Allopurinol, Controloc, Aspirin Protect	Hydroxiurea, Allopurinol, Controloc, Aspirin Protect	Hydroxiurea, Allopurinol, Controloc, Aspirin Protect	Hydroxiurea, Allopurinol, Controloc, Aspirin Protect	Hydroxiurea, Allopurinol, Controloc, Aspirin Protect	Surea, Folacin Aspirin Protect	Surea, Folacin Aspirin Protect	Surea, Folacin Aspirin Protect	Surea, Folacin Aspirin Protect
BM Cellularity	3/4 (75%)	0/1 (0%)	11/16 (69%)	2/2 (100%)	1/2 (50%)	0/1 (0%)	4/5 (80%)	4/5 (80%)	/	1/ 1 (100%)	1/1 (100%)
Follow Up Months	24 (2–218)	113 (11–164)	19 (4–131)	69 (17–159)	40.5 (4–77)	43 (19–102)	90 (68–170)	40.5 (1–100)	109	69	1
Deceased	2/25 (8%)	3/9 (33%)	2/31 (6%)	/	/	/	1/11 (9%)	0/6 (0%)	0/2 (0%)	0/1 (0%)	0/1 (0%)

E-P07.07

Genotype and phenotype of Vietnamese patients with severe combined immunodeficiency

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Introduction: The incidence of Severe combined immunodeficiency (SCID) is about 1:50,000 live births. Genetic mutations can be found in about 85% of patients with SCID. The classification of SCID phenotypes and analysis of gene mutations for the diagnosis has become an important content for treatment.

Materials and Materials and Methods: The study was conducted on 10 patients with diagnosed of SCID. We used sequencing method for screening mutations in 6 genes: *IL2RG, IL7R, RAG1, RAG2, Artemis* and *JAK3*.

Results: 5/10 patients detected mutations in 6 studied genes. 1 patient had homozygous of c.616C>T (p. Arg206Stp) mutation in *IL7Rα* gene with T-B+NK+ phenotype. 3 patients detected mutations in *IL2RG* gene respectively were c.865C>T (p.Arg289Stp); c.272A>G (p. Tyr91Cys) and c.[757G>T;757+1G>C]. All three patients had T-B+NK- phenotype; 1 of this 3 patient received bone marrow transplantation. The chimerism was not increased and patient was died after 1 month of transplantation due to severe infection. We also found 1 patient with compound heterozygous in *RAG2* gene: mutation c.104G>T (p. Gly35Val) inherited from her mother and mutation

c.368G>C (p.Arg123Pro) inherited from her father. This patient had T-B-NK+ phenotype, and she was received bone marrow transplantation at 5 months old. The chimerism test was increase to 100% after 18 days of transplantation.

Conclusions: Identification of disease-causing mutations has important implications in genetic counseling, prenatal diagnosis, treatment and prognosis.

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E-P08
Intellectual disability

E-P08.02
Macroorchidism as a unique sign in 3q13.31 Deletion syndrome

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3q13.31 deletion syndrome is a CNV syndrome which is characterized by marked developmental delay, characteristic faces, hypotonia, and mental retardation. Abnormal genitalia were found in the majority of males, with several having micropenis and also Shawl scrotum, cryptorchidism, small testes. Here we report an interesting case, a 16 years-old male who has machroorchidism with 3q13.31 deletion syndrome. Patient was referred to genetic evaluation due to developmental delay and overgrowth. He was born at 32 weeks of gestation due to premature contractions. Motor milestones delayed; with head control obtained at 6–8 months of age, sitting without support at 12–15 months and walking independently at 24 months old. He was monitored by pediatric cardiologist because of mitral valve regurgitation and once he was hospitalized due to pericardial–pleural effusions after influenza infection. At physical examination he presented with broad forehead, downslanted palpebral fissures, long filtrum, prominent thick lower lip, thin upper lip, and microretrognaty. Parameters of growth were all measured over the highest percentile (weight 71.5 kg (>P99), height 193 cm (>P99) and OFC 60 cm (>p99)) while bilateral orchid volumes were 30 ml. After excluding Fragile X syndrome, microarray analysis was performed and 3,045.807 kb loss at 3q13.2q13.31 (112152600-115198406) region was detected. Congenital deletions affecting 3q13.31 have rarely been reported. There is a hypoplastic male genital dominancy in previously published cases but our case is a new and unique example presented with macroochidism in contrast to the typical form of this syndrome.

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E-P08.03
Association study to determine if there is a phenotypic pattern associated to molecular defects in chromatin remodelling genes

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Introduction: Chromatin regulation has emerged as one of the two domains, along with synaptic function, most affected by causative mutations or Copy Number Variations in neurodevelopmental disorders (NDDs). In this study, we have compared a cohort of 60 patients with syndromic intellectual disability (ID) and variants in genes involved in chromatin regulation, with another cohort of 60 individuals with causal variants in other ID genes. We have evaluated 70 HPO terms to determine if there is a statistical association between some characteristics of the phenotype and molecular defects in genes involved in chromatin regulation.

Materials and methods: We have retrospectively selected 120 patients with syndromic ID evaluated in our Department between 2008 and 2017 with a confirmed molecular diagnosis. Variants were identified by whole exome sequencing (WES) or using a customized NGS panel containing 1586 genes associated with neurodevelopmental disorders (RD-Seq © V4.0). Sanger sequencing was performed for validation of all variants. For the phenotypic statistical association analysis 70 HPO terms were registered and evaluated in both groups.

Results: Results of the phenotypic statistical analysis in both groups and of the causal variants will be presented.

Conclusions: The existence of a phenotypic pattern associated with molecular defects in chromatin remodelling genes might be of clinical interest helping clinicians to recognize the pattern of anomalies and guiding the diagnosis

M. Pacio Miguez: A. Employment (full or part-time); Significant; IdiPaz, HULP.

E-P08.05
Expending the clinical phenotype of CTCF related intellectual disability

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Introduction: Germline heterozygous disease-causing variants in the transcriptional repressor CCCTC binding factor (*CTCF*) gene are a rare cause for a syndromic intellectual disability with cardinal features of short stature, and microcephaly. Thus far, to the best of our knowledge, seven cases were described in the literature.

Case: We present a patient born with cleft palate, dysmorphic facial features, abnormally wide sagittal fissure, atrio-septal defect, mild pulmonic stenosis and low- birth weight. During pregnancy, polyhydramnios and transverse lie were reported. Karyotype and FISH for 22q11.2 chromosome performed after birth were normal. The patient represented to our clinic at 9 years old. She had an intellectual disability, failure to thrive, moderate-severe hearing loss, epilepsy and non-specific changes in brain MRI. In addition, the patient had dental abnormalities including missing teeth. Chromosomal microarray analysis did not reveal clinically significant chromosomal imbalances. Trio whole exome sequencing detected a *de-novo* c.1102C>T; p.Arg368Cys heterozygous variant in the *CTCF* gene, interpreted as likely pathogenic according to ACMG guidelines.

Discussion: Upon comparing the clinical picture of our patient with previously reported individuals (table), some symptoms are shown to be highly repeatable. Microcephaly although common, does not uniformly exist. This case expands the phenotype of CTCF-related disorders, including the presence of brain malformations, epilepsy, and cleft palate as a part of the clinical spectrum. Finely, this is the first case described with hearing loss.

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E-P08.06
Severe intellectual disability and epilepsy due to a parental mosaicism of an *EEF1A2* mutation

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	Our Case	Bastaki F. et al. BMC Med Genet., 2017	Hori I et al. Journal of Medical Genetics, 2017		Gregor A et al. Am J Hum Genet. 2013			
			Case 1	Case 2	Case 1	Case 2	Case 3	Case 4
Global developmental delay and intellectual disability	+	+	+	+	+	+	+	+
Failure to thrive	+	+	−	+	+	−	−	−
Distinct facial characteristics[1]	+	+	+	+	+	+	+	+
Microcephaly	−	+	+	−	+	+	+	−
Cleft palate	+	−	−	−	+	−	−	−
Dental abnormalities	Abnormal in appearance and missing teeth	prominent incisors	−	−	prominent incisors	Dental anomalies reported not specified	−	−
Hearing loss	+	−	−	−	−	−	−	−
Congenital heart malformation	Atrio-septal defect, pulmonic stenosis	Atrio-septal defect, patent ductuc arteriosus	−	−	Atrio-septal defect, patent ductuc arteriosus	−	−	−
Epilepsy	+	−	−	+	−	−	−	−
Neuroimaging	Asymmetry and slight dilatation of lateral ventricles. parenchymal thinning at occipital area	normal	Normal	Normal	ventriculomegaly	Non-available	Normal	Lt ventricle dilatation
Prenatal signs	Intrauterine growth restriction Polyhydramnios, Abnormal lie	Intrauterine growth restriction	Non-available	Non-available	Non-available	Non-available	Non-available	Non-available
Molecular alternation	c.1102C>T; p.Arg368Cys	c.612delAAAG; p. Lys206Profs*13	1.1 Mb deletion of 16q22.1 including CTCF	1.2 Mb deletion of 16q22.1 including CTCF	c.375dupT; p. Val126Cysfs*14	c.1186dupA p. Arg396Lysfs*13	c.1699C>T p. Arg567Trp	280 kb deletion including CTCF

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Introduction: *De novo* pathogenic variants in *EEF1A2* are associated with early infantile epileptic encephalopathy [MIM 616409] and autosomal dominant mental retardation 38 [MIM 616393].

Materials and Methods: A broad gene panel sequencing (*Agilent* 6.110 genes) was performed in a blood sample from our patient followed by parental segregation study. Afterwards, we compare the clinical features with the published cases.

Results: A 15-year-old girl has been observed at our clinical department since she was three years old for etiological investigation of severe intellectual disability, epileptic encephalopathy with myoclonic seizures, autistic behaviour with stereotypies and borderline microcephaly. Additional features included strabismus and sialorrhea. After a previous extensive negative etiological investigation, we found in the *EEF1A2* gene the heterozygous variant c.1295C>T p.(Thr432Met). This variant is not report in the literature or population databases, affects a highly conserved residue and *in silico* analysis predicts deleterious function. It was inherited from the healthy mother who has a low mosaicism (less than 25% by Sanger analysis).

Conclusion: The phenotype in our patient is in accordance with the literature. Until now, only eight *de novo* pathogenic variants have been reported in HGMDPro. Our family is the only known case with a significant risk of recurrence. Our results reinforce the likely pathogenic role of *EEF1A2*-missense variants in intellectual disability, adding more information to the previously described cases.

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E-P08.07

Case report: first case of mosaicism with a premutation post-zygotic retraction in *FMR1* without associated expansion in a male fetus

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Introduction: Fragile X syndrome (FXS) is one of the most common causes of inherited intellectual disability. This X-linked disease is due to abnormal CGG triplet expansion in the *FMR1* 5′UTR (> 200 CGG repeats). Triplet instability during maternal meiosis almost always leads to CGG repeat number expansion, even though allele retraction can occur in less than 1% of the cases. We describe the first case of a post-zygotic premutation retraction without associated expansion, occurring in a male fetus. We discuss mechanisms that could explain this event.

Materials and Methods: The analysis of the number of CGGs was performed using a triplet repeat primed PCR (AmplideX, Theradiag), as part of a prenatal diagnosis in a female carrying a normal allele (29 CGGs) and a premutation (63 CGGs).

Results: The male fetus was free from fragile X syndrome with a somatic mosaic for two different alleles: a 48 CGG grey zone allele and the maternal 63 CGG premutation allele.

Conclusions: Two hypotheses could explain the mosaicism with retraction from a premutation to a grey zone allele:

- a post-expansion retraction hypothesis as proposed by Ferreira et al. with expansion of the maternal premutation followed by a post-zygotic retraction. This hypothesis is highly questionable because the size of the maternal allele is conserved in part of the fetus.
- stability and retraction hypothesis: transmission of the maternal premutation without any CGG expansion followed by post-zygotic retraction to a grey zone allele.

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E-P08.08

Association of epilepsy, antiepileptic drugs and cognitive performance in dizygotic twins with Fragile X syndrome

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Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and autism. Most of FXS cases are due to expansions in the 5′ untranslated region of the *FMR1* gene leading to the absence of the FMRP. It has

been shown that epilepsy occurs in about 20% of patients and that mosaicism is present in approximately 40% of cases. It is reported that dizygotic twinning is more common in FXS permutation carrier females than in general population.

Here we present a case of 13 y.o. dizygotic twin brothers with FXS. Molecular genetic testing showed that one twin has >200 CGG repeats (full mutation) and the other twin has 90 and >200 repeats (size mosaic). Initially mosaic brother used to have better cognitive performance than his full mutation brother, however his development regressed as he developed seizures and was treated with antiepileptic drugs (AEDs). He has been seizure-free for the last five years. The full mutation twin never had seizures and he has better cognitive and behavioral skills. Noteworthy there are two more dizygotic twin pairs in the relatives from the mother's side.

Despite the fact that mosaicism is usually associated with better cognitive functioning, at present mosaic twin brother functions at a lower level than his full mutation brother. Epilepsy and/or AEDs could contribute as an additional impact on cognitive performance in FXS. Presence of twinning in the mother or close relatives of the child with intellectual disability may provide an additional clue for the diagnosis of FXS.

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E-P08.09

Craniofacial appearance of a Developmental Disorder of Chromatin Remodeling in a girl with syndromic intellectual disability and a novel *de novo* microrearrangement encompassing *HNRNPC* gene

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Heterogeneous nuclear ribonucleoproteins (hnRNPs) comprise a large family of RNA-binding proteins (RBPs) involved in multiple aspects of nucleic acid metabolism, including regulation of chromatin remodeling, transcription, RNA stability and splicing, translation, and signal transduction. Mutations in the Heterogeneous Nuclear Ribonucleoprotein K (*HNRNPK*, MIM#616580) gene, encoding a member of the hnRNPs family, have been recently associated with a Kabuki-like syndrome, named Au-Kline syndrome (AUKS, MIM#616580), characterized by facial dysmorphisms, cognitive impairment, and multi-organ defects. Here, we report on a patient with an intellectual disability syndrome, displaying craniofacial anomalies suggestive of a Developmental Disorder of Chromatin Remodeling (DDCR), harboring two *de novo* microrearrangements, consisting of a 4q13.3 microdeletion and of a 14q11.2 microtriplication. The second one includes the Heterogeneous Nuclear Ribonucleoprotein C (*HNRNPC*, MIM#616580) gene, belonging, as well as *HNRNPK*, to the hnRNPs family and nowadays not associated with any genetic disorder. After excluding further underlying molecular defects by family-based exome sequencing, we pointed out *HNRNPC* as a probable contributor to the DDCR craniofacial appearance in our patient, through an altered gene dosage. *HNRNPC* encodes heterogeneous nuclear ribonucleoprotein C1/C2, a ubiquitously expressed RNA-binding protein (RBP), that influences various aspects of mRNA metabolism and intervenes in the ATP dependent chromatin remodeling process. Moreover, we performed a DeepGestalt analysis trough the Face2Gene platform, confirming an overlap between facial dysmorphisms of our proband and patients harboring *HNRNPK* mutations. This case highlights and corroborates the importance of an emergent subgroup of conditions, in the context of DDCRs, caused by anomalies in the hnRNPs components.

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E-P08.10

Heterozygous *IGF1* deletion in a family with short stature, microcephaly and intellectual disability

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Introduction: Frequently, the underlying etiology of short stature is unknown, resulting in a diagnosis of idiopathic short stature.

Case report: We present a family with short stature: the proband, a 15 months old child, her 34 years old mother and maternal grand-mother. The phenotype among them is slightly different, as the child and her mother have also

microcephaly and developmental delay/learning disabilities, respectively. Maternal medical history was remarkable for intrauterine and postnatal growth restriction. The diagnosis of Russel-Silver syndrome was suggested and she received growth hormone therapy from 9 to 15 years, achieving a final height of 149 cm. From 16 to 22 years of age she was treated for anorexia nervosa and at 31 years of age insulin resistance was diagnosed.

Results: Cytogenetic analysis and MLPA studies for *SHOX* gene deletions and BWS/RSS del/dup/IC defects were normal in the mother. The aCGH identified a 200Kb deletion at 12q23.2 close to *IGF1* gene, not present in the maternal grand-mother. MLPA analysis confirmed the heterozygous loss of exons 1 and 2 of *IGF1* in the mother and the proband.

Conclusions: Insulin-like growth factors (IGFs) are important for growth and development. Homozygous *IGF1* deletions or mutations are relatively rare. The phenotypic severity is variable and includes intrauterine growth retardation, postnatal growth failure, microcephaly, varying degrees of developmental delay, sensorineural hearing loss, and hyperinsulinism. A few patients with heterozygous mutations have been described mainly with only short stature. Here we describe two patients bearing a heterozygous *IGF1* deletion with an expanded phenotype.

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E-P08.11

Definitive diagnosis of a female with speech delay using clinical exome sequencing: a rare case of Coffin-Lowry syndrome

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Introduction: We present a female in whom targeted clinical exome sequencing supported definitive diagnosis of a rare hereditary disorder.

Materials and Methods: A 3-year old girl was referred to the Department of Medical Genetics, Athens University with speech delay and abnormal facial features. Laboratory genetic investigation involved classical karyotype analysis (negative), followed by semi-targeted Exome Sequencing, 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). Bioinformatic data analysis was evaluated with: *SOPHiA DDM*® (Sophia Genetics) and VarAFT 2.14.

Results: A novel heterozygous pathogenic variant NM_004586.2:c.932T>G, p.(Leu311*) was identified in the *RPS6KA3* gene on the X chromosome, associated with Coffin-Lowry syndrome.

Discussion: The *RPS6KA3* gene provides instructions for making a ribosomal-S6 kinases (RSKs) that play role in several important cellular processes, cell growth, proliferation, differentiation and apoptosis. More than 125 mutations in the *RPS6KA3* have been identified in people with CLS. These mutations severely reduce or eliminate the activity of the RPS6KA3 protein. Coffin-Lowry syndrome is a rare form of semi-dominant X-linked mental retardation, as it usually presents more severe expression in male than in female individuals. The majority of CLS females have only minimal findings such as mild facial coarsening, obesity and tapering fingers with normal intelligence or mild learning difficulties. CES can support definitive diagnosis in patients with speech delay of unknown etiology, contributing to precise prognosis, systematic monitoring, and when appropriate, identification of other family members.

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E-P08.12

Novel genomic rearrangements affecting chromosome 15 in Bulgarian cases with epilepsy and intellectual disability

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High resolution chromosomal microarray (CMA) is now worldwide first tier diagnostic tool for detection of submicroscopic Copy Number Variations (CNVs), as genetic cause of neurodevelopmental disorders. Recently, it became evident that the co-occurrence of epilepsy, autism, attention deficit and hyperactivity disorder (ADHD) and intellectual disability (ID) is related to common biological pathways and in this sense, one CNV may be

causative for more than one neuropsychiatric phenotype. In the present study, we performed array Comparative Genomic Hybridization (aCGH) using Agilent Microarray Kit, 4x180K in Bulgarian patients with comparable neurodevelopmental disorders such as epilepsy, ID and autistic features. A quantitative PCR was conducted for confirmation and segregation analysis. As a result, we detected one novel deletion 1.33 Mb in size and 402-kb duplication covering different sub—bands of chromosome 15 in two non-consanguineous patients with unprovoked seizures, variable severity of ID, behavior problems and congenital anomalies. The deletion is located in 15q22.31 region and covers 16 OMIM genes. Among them, *CSNK1G1* gene is most likely associated with the seizure phenotype, observed in our patient. The duplication is located in 15q13.1 region, covers the whole *APBA2* gene and was found in three generations of a family with epilepsy, ID and psychiatric abnormalities. This study demonstrates the importance of CMA as comprehensive genomic approach for identification of novel microaberrations responsible for neurodevelopmental disorders. The study was supported by Grants DTK/67/2009 and DUNK 01-2/2009 of NSF, Ministry of Education and Science, Bulgaria.

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E-P08.13
How new technologies can improve the old clinical awareness?

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Introduction: Intellectual disability is a neurodevelopmental dysfunction with onset during developmental period that includes both intellectual and adaptative functioning in conceptual, social and practical domains. Historically, a specific diagnosis has been made achievable in only a minority of the cases, but this has changed dramatically with the use of new diagnose technologies.

Materials and Methods: Clinical and molecular characterization of three cases with intellectual disability and non-specific dysmorphisms in our hospital, including retrospective review of previous patients described in literature with pathogenic variants.

Results: Our three patients had a specific diagnosis identified after perform a commercial broad disease gene panel. All of them had previously an extensive etiological investigation. We found in patient 1 a probable pathogenic frameshift variant in the *ZMYND11* gene: c.1317_1320del [p.Thr440Argfs*3]; in patient 2 a pathogenic missense mutation in the *PPP2R5D* gene: c.592G>A [p.(Glu198Lys)]; and in patient 3 a probable pathogenic frameshift variant in the *STXBP1*: c.1099C>T. They were *de novo* in patients 2 and 3 and inherited from an affected parent in patient 1.

Discussion: The search for a specific diagnosis is a key component of the assessment of the child with intellectual disability. These cases demonstrate how the new technologies can change the number of cases in which we can achieve a specific diagnosis, that had previously escaped in clinical recognition.

In retrospective, after reviewing the literature, it is possible that patient 1 and patient 2 have recognizable phenotypes and this could alert us to suspect of these specific causative genes in future cases.

P. Maia Almeida: None. **J. Sá:** None. **F. Ramos:** None. **J.M. Saraiva:** None.

E-P08.14
Epilepsy in patients with intellectual disability

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Introduction: Epilepsy as a neurological disorder has deep influence on patient life. The incidence among individuals with intellectual disability is higher than in general population—22 vs. 0.6–1%, respectively. Here we present results from a study of Bulgarian patients with co-occurring epilepsy and intellectual disability.

Materials and Methods: Ten patients with sporadic early onset epilepsy and intellectual disability were tested through NGS (Massive parallel sequencing) on an Illumina platform using TruSight One gene panel and/or WES. Previous genetic tests of *SCN1A* were negative in these patients and microstructural chromosome abnormalities have been ruled out.

Results: Four different probably causative mutations were found: heterozygous mutations in the *SCN8A* gene

(NP_055006.1:p.Arg1872Gln) and in the *SCN2A* gene (NP_001035232.1:p.Arg853Gln); a hemizyoguos mutation in *SLC9A6* (NP_001036002.1:p.Arg500Ter); and an unreported heterozygous mutation in *MTOR* gene (NP_004949.1:p.Val2406Met). Among parents of affected individuals no carriers of mutant alleles have been observed.

Conclusion: To establish the genetic basis of a heterogeneous disorder as epilepsy with intellectual disability could be challenging and complex. The higher incidence of epilepsy among patients with intellectual disability than in general population suggests that both disorders may possess a partially shared genetic background. Results from our pilot study show that in some cases severe early onset disorders like epilepsy and mental retardation could result from de novo dominant single gene defects, which hampers their prophylaxis. Despite this, knowledge of the underlying genetic cause is helpful in genetic counseling of patients and may point out new targets for treatment.

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E-P08.15

New homozygous missense variant in *KDM5B* gene - guilty unless proven innocent

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Introduction: In 2018 Faundes et al. described three cases of a new autosomal recessive developmental delay (DD) syndrome (MRT65; MIM 618109) caused by bi-allelic loss of function (LoF) variants in *KDM5B* gene. All three patients had severe DD, camptodactyly and overlapping facial dysmorphism. KDM5B belongs to the KDM5 family of JmjC domain-containing histone demethylases which specifically remove dimethyl and trimethyl marks from histone 3 lysine 4 (H3K4), acting as transcription modulators.

Case Presentation: An 8-year-old boy was referred for genetic evaluation for DD and cataplexy. He was the second child of consanguineous parents; his father and sister had febrile seizures, and one of his maternal uncles had cerebral palsy. Congenital torticollis and bilateral cryptorchidism were noted in the neonatal period. He evolved with moderate to severe DD, absence of speech, hypotonia, joint hyperlaxity, cataplexy triggered by laughter and tickles, attention deficit disorder, astigmatism and hypogonadism. Brain MRI showed white matter reduction and ventricular asymmetry. On physical examination he had a square face, hypertelorism, epicanthus, broad nose, macrotia, and small hands with tapering fingers. Karyotype, *FMR1* molecular analysis, *RPS6KA3* (*RSK2*) gene sequencing, and array-CGH were normal.

Results: Whole exome sequencing was performed revealing a homozygous *KDM5B* missense variant: c.1976G>A, p.(Arg659Gln). Homozygosity was confirmed by segregation analysis. This variant was previously unreported and is classified as of uncertain clinical significance.

Conclusions: This patient has similar features to those reported before with bi-allelic LoF *KDM5B* variants. Assuming his variant is causative, he contributes to expand the phenotypic spectrum associated with KDM5B deficiency.

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E-P08.16

Novel compound heterozygous splicing variants in *KIAA1109* broaden the mutational spectrum and refine the clinical picture of Alkuraya-Kucinskas syndrome (ALKKUCS)

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Biallelic loss of function (LoF) and missense mutations in *KIAA1109* (OMIM *611565) encoding an evolutionary conserved protein with yet poorly understood function are described as causative of the recently characterised Alkuraya-Kucinskas Syndrome (ALKKUCS, OMIM #617822). Patients with this rare autosomal recessive and often lethal disorder generally share a clinical picture of profound developmental delay, infantile epilepsy, ventriculomegaly, cerebellar hypoplasia, cerebral parenchymal rarefaction and arthrogryposis. So far, only 13 patients described in a single report (Gueneau et al., 2018) and two prenatally identified fetuses (Filatova et al., 2018) have been published with a spectrum of severity ranging from death in utero or soon after birth in association with LoF mutations to several neurodevelopmental disease and brain anomalies in individuals with missense variants compatible with life. Using exome sequencing we identified a boy born to healthy non-consanguineous German parents with severe neurodevelopmental delay, hypotonia, Dandy-Walker malformation, hydrocephalus, cerebral atrophy, epilepsy,

respiratory insufficiency, hypothyroidism and dysmorphic facial features but no arthrogryposis and *KIAA1109* compound heterozygosity for a nonsense and a splice variant inducing skipping of an exon (confirmed by RT-PCR). Through comparison with the hitherto described patients and characterisation of the splicing mutation in this patient we expand and refine the phenotypic and mutational spectrum of ALKKUCS.

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E-P08.17

A *de novo* balanced 3;5 translocation in a patient with intellectual impairment, autistic features, macrocephaly and absence epilepsy putatively affects *MEF2C* expression

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Haploinsufficiency of *MEF2C* is known to cause severe developmental delay, intellectual impairment, autistic features and epileptic encephalopathy.

We report a 4-year-old female patient with severe intellectual impairment, macrocephaly, autistic features and absence epilepsy. She presented with developmental delay from birth, has no expressive language and is not able to walk. CMRI was unremarkable. The girl carries a *de novo* balanced translocation, with the karyotype 46,XX,t(3;5)(p22.1;q14.3). Array CGH analysis and trio exome sequencing did not reveal any copy number variation and/or sequence variant of pathogenic relevance.

By using serial fluorescence in situ hybridization (FISH), we narrowed down the breakpoint in 3p22.1 to ~850 kb. No genes of putative clinically relevance have been described within this chromosomal region. The BAC clone RP11-690G22 spanned the 5q14.3 breakpoint. We delineated the breakpoint to ~50 kb; it mapped either in the proximity of the MEF2C gene or directly disrupted this gene. Two patients with a *de novo* translocation and a 5q14 breakpoint in proximity to *MEF2C* have been reported in the literature (Floris et al. 2008, Saitsu et al. 2011). Both had clinical features typically reported for individuals with *MEF2C* haploinsufficiency.

Our data suggest that the chromosomal rearrangement in our patient leads to deregulated *MEF2C* expression likely underlying the clinical features in the girl.

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E-P08.18

A duplication of the whole *MID1* gene in a 14-years old boy with a non-Opitz GBBB phenotype

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The *MID1* gene (Xp22.2) spans approximately 400 kb of genomic sequence and encodes the MID1 protein, a central enzyme involved in many different processes in the cell and a member of the RING (Really Interesting New Genes) finger family. Besides regulating the activity of the translational regulators PP2A (protein phosphatase 2A) and mTOR (mammalian target of rapamycin), MID1 assembles a large microtubule-associated protein complex involved in mRNA transport and translation. Thus, it exerts the role of an E3 ubiquitin ligase and is a central player during development. A spectrum of *MID1* mutations comprising missense mutations, nonsense mutations, small insertions and deletions, splice site alterations as well as exon deletions and duplications, were found to cause the X-linked form of Opitz G/BBB syndrome (XLOS, OMIM 300000), an entity with a very distinguished phenotype.

Here we report on a 14-years-old boy with a novel maternally inherited duplication, 550 bp in size, comprising the whole *MID1* gene. Remarkably, patient's phenotype differed considerably from the one delineating XLOS. He presented with mild to moderate ID, hypermetropia, febrile seizures between the age of 9 months and 5 years, as well as the following minor anomalies: long narrow face, hypotelorism, upslanting palpebral fissures, blepharophimosis, prominent base of the nose, long prominent nose, low hanging columella, wide mouth, upturned corners of the mouth, micrognathia, posteriorly rotated ears and macrotia. His gestalt shares features between Schillbach-Rott syndrome and Floating-Harbor syndrome. Single exome analysis including *SRCAP* gene did not find any disease-causing gene variants. Thus, patient's diagnosis remains unclear.

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E-P08.19

Severe neurodevelopmental disease caused by a homozygous *TLK2* variant

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Introduction: *TLK2* encodes the Tousled-like kinase 2, a nuclear serine/threonine kinase known to be involved in DNA replication and chromatin assembly. *TLK2* has recently been associated with a distinct neurodevelopmental phenotype characterised by mild motor and language delay, behavioural problems, facial dysmorphism and gastro-intestinal symptoms. All 40 individuals reported carried heterozygous *de novo* or dominant variants in the *TLK2* gene; truncating variants located throughout the gene and missense changes principally located at the C-terminal end of the protein.

Methods: Patient and family members were recruited at the Department of Paediatric Neurology, Izmir, Turkey. Whole exome sequencing (WES) was performed at the Broad Institute, USA using Illumina exome capture (38 Mb target). WES data were analysed on the RD-Connect Genome-Phenome Analysis Platform (https://platform.rd-connect.eu/). Likely pathogenic variants were identified applying standard filtering criteria: MAF <1%, and high to moderate variant effect predictor.

Results: We identified a homozygous missense variant (c.163A>G; p.Lys55Glu) in *TLK2* in a girl with a neurodevelopmental disorder with severe motor and language delay, behavioural problems, facial dysmorphism and gastro-intestinal symptoms, in keeping with what has been described for heterozygous *TLK2* patients. Our patient however present more severe symptoms, with profound ID, West syndrome, pontocerebellar hypoplasia and spastic tetraparesis. Both parents are heterozygous for the variant and clinically unaffected. Our variant is located at the N-terminus in a region expected to contain a nuclear localization signal.

Conclusion: This report highlights that recessive variants in *TLK2* can also be disease-causing and may act through a different pathomechanism.

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E-P08.20
NRXN3 end-duplication in not sufficient to explain development delay

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Neurexins are presynaptic proteins that act as synaptic organizers, play a role in synapse functions and conduction of nerve signals. The neurexin family consists of three genes (*NRXN1, NRXN2* and *NRXN3*) in whom genomic alterations have been identified in variable neuropsychiatric disorders. Nevertheless, regarding the incomplete penetrance and the variability of the symptoms associated with Lof mutation of neurexin genes, authors have suggested implication of others genetic risks modulating the neurodevelopment. We reported a patient with feeding difficulties, psychomotor delayed, limited speech. CGH array identifies a 150kb duplication at 14q31.1 including the last two exons

of both alpha and beta isoforms of *NRXN3*. Interestingly, in DECIPHER database, the patient 257388 carried a similar duplication inherited from an asymptomatic parent, as our patient. Suggesting that the duplication interrupting *NRXN3* is not enough to explain his phenotype, additional genetic investigations were carried out and clinical exome sequencing identified a *de novo ASXL3* nonsense mutation (p.Ser1441*) consistent with diagnostic of Bainbridge-Ropers syndrome. This developmental disorder has been described in around 22 patients to date. The variable phenotype is associated with intellectual disability, poor speech, autistic traits, distinct face and significant feeding difficulties compatible with our patient phenotype. Our observation suggests that the *NRXN3* end-duplication could contribute to modulate the phenotype of our patient but is likely not sufficient to explain it. This report highlights difficulties to interpret risk factor variants in neurodevelopmental disorder and the necessity to perform additional genetic testing such as exome sequencing in order to exclude others rare pathogenic variants.

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E-P08.22
Mutation in the PPP2R5D gene in a patient with macrocephaly and developmental delay - Jordan´s syndrome

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Introduction: Recent researches showed high proportion of mutations in the PI3K-AKT-mTOR pathway (*PTEN, AKT1, AKT3, PIK3CA, MTOR, PIK3R2, CCND2, PPP2R5D, PPP2R1A* genes) in patients with macrocephaly and developmental delay/ autism spectrum disorder. The *PPP2R5D* gene codes a protein called B56-delta, a subunit of the enzyme phosphatase 2A (PP2A). PP2A dephosphorylates proteins in PI3K-AKT-mTOR pathway and plays an important role in neuronal and developmental regulation processes. *PPP2R5D*-related disorders are inherited in an autosomal dominant manner but mutations mainly occur de novo.

Case: Our patient was born from oocyte donation assisted pregnancy, at term, by caesarean section due to prenataly evaluated macrocephaly. At birth his head circumference was +2.2 SD, BW and BL at 90th percentile, AS were 8/9. Beside the macrocephaly, hexadactyly, hypotonia and mild dysmorphic features, such as frontal bossing, hypertelorism, small chin were observed. Psychomotor development has been delayed since the birth. Brain MRI showed periventricular changes due to perinatal hypoxic/ischemic injury. ArrayCGH was normal as well as metabolic assessment. WES showed a heterozygous pathogenic variant in the *PPP2R5D* gene: c.592G>A(p.Glu198Lys). This missence mutation correlates with patient`s clinical findings. A heterozygous variant in *CHD4* gene was also found, the clinical relevance is unknown.

Conclusion: It is important to consider mutations in the PI3K-AKT-mTOR pathway in the evaluation of children with macrocephaly and developmental delay. *PPP2R5D*-related conditions are rare, so far there are about 70 diagnosed patients around the world. New researches focusing on the role of the *PPP2R5D* gene in intellectual disability, autism, Alzheimer's disease and cancer are promising.

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E-P08.24
Acute encephalopathy after head trauma in a patient with a RHOBTB2 mutation

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Background: De novo missense mutations in the *RHOBTB2* gene have been described as causative for a developmental and epileptic encephalopathy. The clinical phenotype of this disorder includes early-onset epilepsy, severe intellectual disability, postnatal microcephaly and movement disorders. Three *RHOBTB2* patients were described with acute encephalopathy and febrile epileptic status. All showed severe EEG abnormalities during this episode and abnormal magnetic resonance imaging with hemisphere swelling or reduced diffusion in various brain regions.

Patient: We describe the episode of acute encephalopathy in a patient with a RHOBT2 mutation after head trauma. At admission to the hospital he showed a E4M4V1 Glasgow coma scale score. EEG was severely abnormal showing a non-continuous pattern with slow activity without epileptic activity indicating a severe encephalopathy. A second EEG on day 8 was still severely slowed and showed focal delta activity fronto-centro-temporal in both hemispheres. Gradually he recovered, and on day 11

he had regained his normal reactivity, behavior and mood. Two months after discharge, a new EEG was made showing a further decrease in slow activity and increase in normal electroencephalographic activity. After discharge, parents have noted that he shows more hyperkinetic movements compared to before this period of encephalopathy. Follow-up MR imaging showed an increment of hippocampal atrophy.

Conclusions: Acute encephalopathy in RHOBTB2 patients can also be triggered by head trauma.

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E-P08.25

RORB mutation in two sisters with Epileptic Encephalopathy with Continuous Spike and Wave During Sleep (CSWS)

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Introduction: Recent year's access to next generations sequencing enable to detect a great number of causes to genetic generalized epilepsies. *RORB*, located in 9q21.13, encodes the nuclear receptor RORβ. Two isoforms of RORβ are found in humans—RORβ1 and RORβ2. RORβ1 is expressed in cerebral cortex layer IV, thalamus and hypothalamus while RORβ2 is the predominant isoform in retina and pineal gland and participate in control of circadian rhythmicity. RORβ1 is considered to play an important role in thalamocortical circuitry where disturbance can generate generalized seizures. We report a previously undescribed mutation in *RORB* in a family with two sisters with epileptic encephalopathy with CSWS.

Methods: We performed whole exome sequencing and analyzed a gene panel including 917 genes reported in epileptic encephalopathy or inborn metabolic disease in the two siblings and their parents. Mutations were verified by Sanger sequencing.

Results: We detected a heterozygote missense mutation in *RORB*, c.1090T>C, (p.Ser364Pro) in both sisters. The mutation is not previously described in healthy controls or epileptic encephalopathy. Polyphen2 and SIFT predict the mutation as being benign or pathogenic. The same mutation was detected in asymptomatic father. This could indicate a variable penetrance of the mutation since a paternal aunt and uncle have epilepsy since childhood and intellectual disability. Unfortunately they were unavailable for genetic testing.

Conclusion: Here we present a possible new mutation and phenotype of *RORB* characterized by pharmacoresistant epileptic encephalopathy with CSWS, speech impairment and clear cognitive decline leading to mild intellectual disability.

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E-P08.26

A supernumerary marker chromosome 15 (SMC15) in 8-years old boy with intellectual disability, developmental delay and autism spectrum disorders

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Introduction: Deletion of 15q11.2-q13 results in either Prader-Willi (PWS) or Angelman syndrome (AS) depending upon the parent of origin. Duplications of the Prader-Willi-Angelman critical region (PWACR) are rare and associated with behavioral and neurological features and also depend on the parent of origin. According to multiple reports maternal duplications/triplications are pathogenic, while pathogenicity of paternal duplications is still controversial. We present an 8-years old boy with triplication of 15q11-13 (PWACR) of maternal origin.

Methods: We exploited methods like: array comparative genomic hybridization (aCGH 180k+SNP), G-band karyotyping, FISH with 15q11.2 (SNRPN/IC) and acro-p-arm satellite probes and methylation-specific MLPA analysis (MS-MLPA).

Results: An 8 year old boy was referred to us for genetic testing because of psycho-motor delay, mental retardation and dysmorphic features. High-resolution aCGH showed a large triplication (9.5 Mbp) of PWACR (15q11.1-q13.1). Further performed MS-MLPA detected that the boy had one copy of paternal while three copies of maternal PWACR. Karyotyping with FISH carried out from peripheral blood lymphocytes detected a supernumerary marker chromosome 15 (SMC15) in mosaic state (60%) which came out to be consisted of two copies of PWACR.

Conclusions: Duplication or triplications of PWACR (15q11-q13) are associated a distinct phenotype which has some sharing features of both PWS and AS, while it is well

known that maternal interstitial duplications/triplications are pathogenic, pathogenicity of paternal duplications is still equivocal. Additionally, we conclude that a karyotyping should always be performed in all patients with duplication or triplications of Prader-Willi-Angelman critical region to not miss any supernumerary marker chromosomes.

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E-P08.27

A case of different penetrance of *FMR1* resulting in varying phenotype among three brothers who are offspring of a premutated mother

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Introduction: Fragile-X syndrome, an X-linked dominant disorder readily manifested in affected males, is a well characterized form of inherited mental retardation. Number of repeats of CGG in the 5′ untranslated region of *FMR1* at Xq27.3 ranges from common (5–44), intermediate (45–54), premutation (55–200) to full mutation (>200) and causes varying cognitive, behavioral and physical phenotype.

Materials and Methods: A 3-year old boy showing distinctive Fragile-X traits (global developmental delay, intellectual disability, hyperactive behavior, autism spectrum disorders, long and narrow face, large ears, prominent jaw and forehead) appeared in our laboratories and was tested for *FMR1* using FRAXA specific primers and MS-MLPA to test methylation status. His younger 1-year-old brother was also tested due to physical malformations only while his older 5-year-old brother showing no apparent physical and mental abnormalities as well as the parents were also screened.

Results: The mother owned a premutated allele of 83 and a normal allele of 39 repeats. The son with the severe phenotype had more than 300 repeats while the son showing only physical malformations had more than 200 repeats. Both boys owned methylated CpG islands. The third son as well as the father had normal alleles (<40 repeats).

Conclusions: The family described is one of the least reported cases that manifest the impact of genetic penetrance which in the case of Fragile-X syndrome depends on the CGG repeats as well as the methylation status of *FMR1*. The stochastic result of X reactivation resulting in normal offspring is also well shown.

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E-P08.28

Microcephaly and intellectual disability with novel compound heterozygous WARS mutations

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Introduction: Mutations in genes encoding aminoacyl-tRNA synthetases are known to cause leukoencephalopathies in autosomal recessive (AR) fashion. Mutations in some aminoacyl-tRNA synthetases are associated with autosomal dominant (AD) inherited form of axonal neuropathy. *WARS* encodes the human cytoplasmic tryptophanyl-tRNA synthetase. Tsai et al. reported a recurrent p.His257Arg mutation in *WARS* in three unrelated autosomal dominant distal hereditary motor neuropathy pedigrees. We identified novel compound heterozygous *WARS* mutations by Whole exome sequencing (WES) in a patient with microcephaly and intellectual disability with delayed myelination.

Clinical Report: The 2-year-old female was the first child of healthy and non-consanguineous Japanese parents. She was hypotonic and her developmental milestones were markedly delayed. Brain MRI revealed delayed myelination. She was suspected to have congenital CMV infection or Pelizaeus-Merzbacher syndrome. But they were excluded. Physical examination identified microcephaly (-3SD). Independent walk was not possible. She spoke no meaningful words. Recently, she experienced a generalized seizure at 2 years old.

Method: With the approval of our institutional ethics committee, DNA samples from the patient and her parents were analyzed by WES.

Results: The patient was compound heterozygous for WARS. (NM_004184: exon11:c.C1342T:p.R448W, exon9: c.G997A:p.A333T.) Her parents were heterozygous for the

mutation. Both mutations were novel and predicted to be pathogenic *in silico* analyses.

Discussion: Mutations in aminoacyl-tRNA synthetases may cause neuropathy in AD fashion and encephalopathy in AR fashion. We suppose that abnormality of *WARS* causes a novel neurogenetic syndrome with microcephaly and intellectual disability with delayed myelination. Our findings broaden the genetic and clinical spectrum associated with *WARS* mutations.

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E-P08.29

Phenotype of the first patient harboring a *de novo* in-frame deletion in the LisH (LIS1 homology) domain of the *WDR26* gene

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Background: *WDR26* haploinsufficiency was identified as cause of a distinct syndromic entity associated with intellectual disability and significant language impairment, (non-)febrile seizures, gait abnormalities, as well as a distinctive facial phenotype (MIM #617616). Hitherto, only 15 *WDR26*-affected individuals have been reported, among which two patients harbored a *WDR26* missense variant in the CTLH (C-terminal LIS-homology) domain, which is prototypical for the WD40-protein class aside from the WD40 repeats and the LisH-domain.

Case Presentation: For the first time, we identified a *de novo* in-frame deletion [c.439_441del p.(Leu147del)] within the *WDR26* LisH-domain in a boy presenting with neurodevelopmental delay associated with profoundly impaired expressive language and similar facial features as the previously published patients. Further prominent features were transient hyperactivity, autistic-like behavioral problems, and a history of grand mal seizures and several drop attacks. In comparison to the literature, our propositus additionally shows a decreased pain sensation and a hyperlordosis; at ECG evaluation, a mild dilatation of the ascending aorta was found. Interestingly, unlike the *WDR26*-patients published before, he does not present with gait abnormalities. Considering new available structural data by Ulrich et al. (Structure 2016), the detected deletion is likely to interfere with the WDR26 domain structure and extrinsic interactions as it was postulated before for the four hitherto known *WDR26* missense variants by Skraban et al. (AJHG 2017).

Conclusion: This first presentation of a patient with a *de novo* in-frame deletion in the *WDR26* LisH domain may substantiate further investigations about potential genotype-phenotype correlations and the pathomechanisms of *WDR26* haploinsufficiency.

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E-P08.30

A novel de novo frameshift variant identified in *AHDC1* in two unrelated boys with Xia-Gibbs syndrome

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Background: Xia-Gibbs syndrome (XGS) is a neurodevelopmental disorder characterized by intellectual disability, hypotonia, developmental delay, sleep apnoea and mild facial dysmorphism. Heterozygous loss-of-function variants in *AHDC1*, encoding the AT-hook DNA binding motif containing 1 transcription factor, was discovered in 2014 as likely causative of XGS. To further delineate the complex genotype-phenotype relationship of this disorder we present two unrelated cases of XGS caused by a shared novel frameshift variant: c.2849del (p.(Pro950Argfs*192)) in *AHDC1* identified by whole-exome sequencing.

Case description: Following a normal pregnancy and uneventful delivery at term, proband 1 presented at the age of 8 weeks with severe hypotonia and mild facial dysmorphism with anteverted, narrowed nares and a tent-shaped, myopathic mouth. Subsequently the boy displayed reduced motor skills and was only able to communicate via nonverbal language. MRI demonstrated thinning of corpus callosum and low-grade periventricular leucomalacia. The patient had surgery for bilateral cryptoorcihdism. Interestingly, a facial lipoma and bilateral calcaneal varus foot deformity was noted, which may be unrecognized manifestations of XGS. Proband 2 presented with hypotonia, delayed psychomotor development and subtle dysmorphism in early infancy. At the age of 6, he was diagnosed with infantile autism. He was diagnosed with epilepsy at the age of 7. MRI of the cerebrum was normal.

Conclusion: This case report illustrates the clinical heterogeneity of two cases of XGS that share the same causative variant, which suggest that additional genetic or environmental modifiers could be at play in this disorder.

Larger cohort studies are warranted to further disentangle these questions.

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E-P08.31
Recurrence risk for Xia-Gibbs syndrome caused by an apparent *de novo* variant in *AHDC1* is explained by low level maternal mosaicism

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Introduction: Xia-Gibbs syndrome is characterized by hypotonia, intellectual disability, global developmental delay, mildly dysmorphic facial features and apnea. All patients reported to date carry truncating *de novo* variants in *AHDC1*. Recurrence risk for Xia-Gibbs syndrome is therefore considered negligible.

Materials and Methods: Whole exome sequencing (WES) of a patient with a complex, presumably genetic condition was performed in a routine diagnostic setting. Confirmation of a candidate causative variant as well as determination of the parents' genotype for this variant was based on targeted Sanger sequencing.

Results: The main clinical findings in our patient include severe intellectual disability, psychomotor retardation, stereotypic movements and autistic behavior. Global developmental delay and cerebral palsy were reported for a sibling, who was not yet available for detailed clinical and genetic work-up. WES identified the *AHDC1* frameshift variant c.1338del (p.(Ser447Glnfs*5)) in the index. The variant was not found in the father, but Sanger sequencing traces were suggestive of very low level mosaicism in the mother. Repeated analysis on an independent secondary sample confirmed this observation.

Conclusion: Our findings may represent the first instance of recurrence of Xia-Gibbs syndrome. Testing of this hypothesis has been initiated. Parental mosaicism for apparent *de novo* variants may be an underestimated cause for phenotypes which, at first glance, seem to be inherited in a recessive manner.

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E-P09
Neurogenetic and psychiatric disorders

E-P09.01
Copy number variants in Alzheimer's disease

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Introduction: Alzheimer's disease (AD) is a devastating neurological disorder primarily affecting the elderly people, manifests by progressive deterioration and loss of memory. Despite the identification of nearly 40 common genome-wide significant risk loci in genome-wide association (GWAS) and whole exome sequencing (WES) studies in case-control cohorts, the marginal joint effect of all known loci accounts only for a fraction of the estimated heritability of AD suggesting that the major genetic contributors to the disease risk have not been identified yet or that other forms of genetic variation such as copy number variations (CNVs) might be playing a role. Indeed, several studies have shown that susceptibility to neurological phenotypes of complex disease such as autism, schizophrenia, AD and bipolar disorders is linked to the presence of CNVs.

Methods: The aim of this study is to identify any potential CNVs candidate regions in AD using data from a unique cohort of clinically characterized and neuropathologically defined 5469 Caucasian sporadic and healthy control participants (4386 AD cases (1911 EOAD and 2475 LOAD) and 1083 controls). WES data has been generated using Illumina TruSeq Rapid Exome Library Prep Kit and sequenced on Illumina HiSeq 4000 at a median coverage of 20X. We will be evaluating the detection of clinically relevant, rare de novo CNVs of varying size and copy number state using both CLAMMS and WISExome tools.

Results: We will be presenting our first large scale copy number variants analysis results based on the WES data for the cohort at the ESHG2019.

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E-P09.02

The frequency of microsatellite polymorphism (RS1) of arginine-vasopressin gene *AVPR1A* in the Yakut population

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Genetic variations in of the arginine-vasopressin receptor (AVPR1A) can cause the formation of individual personality traits and temperament, characterized by an increased desire for new experiences, impulsiveness, sociability, levels of neuroticism and extroversion. In this report, a frequency of microsatellite polymorphism (RS1) of arginine-vasopressin gene *AVPR1A* in the Yakut population (334 healthy individuals) was carried out. Alleles of this marker with the number of repetitions from 8 to 9 are presented in different populations. We have identified eight different alleles of the RS1 marker (R8, R9, R10, R11, R12, R12, R13, R14, R15). Allele frequency distribution in all populations is generally characterized by single-modality with predominance of allele frequency R10. Short and long alleles (R8, R14, R15) are absent in our sample in homozygous form. Due to the high frequency of allele R10 (usually more than 40%, in our sample 45.3%) RS1 is allocated the lowest level of heterozygosity among the studied loci. The Yakut population is characterized by a pronounced deficit of heterozygotes and a low level of actual heterozygosity, which can be explained by the founder's effect in this population. This study was supported by the Ministry of Education and Science of the Russia (#6.1766.2017) and the RFBR (18-05-600035_Arctica).

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E-P09.03

Clinical description of a male patient with tall stature, intellectual disability, and seizures with an ARHGEF9 deletion

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Background: The phenotype of patients with copy number variants in region Xq11.1q11.2 including the gene *ARHGEF9* remains elusive given the scarcity of cases described. This case report describes an adult patient with a deletion on Xq11.1q11.2, presenting tall stature, intellectual disability and uncontrolled seizures.

Case: A male patient presented from birth macrosomia and hypotonia. At 14 months of age, he had the first episode of seizures. His psychomotor development was delayed from the early neonatal period, starting independent walking at age 8 and never acquiring intentional expressive communication. At age 21, he has severe intellectual impairment, daily seizures refractory to treatment (without an identifiable trigger), progressive paraparesis, multiple stereotypies, polyphagia, constipation, severe scoliosis, and valgus knee. His height is above the 99 percentile (P) (z= +3.9), and head circumference P91-98. On his brain MRI it was described abnormal hippocampal rotation, mega cisterna magna and a pineal cystic formation. Now he is under a trial with ketogenic diet to control his seizures. On the arrayCGH analysis, a de novo deletion of the Xq11.1q11.2 region was detected, including the gene *ARHGEF9*. Pathogenic variants causing haploinsuficiency of this gene are associated with epileptic encephalopathy, early infantile 8 (MIM 300607), a form of epilepsy with poorly controlled and hyperekplectic seizures, the latter not present in this patient.

Conclusion: The scarcity of cases described with *ARHGEF9* haploinsufficiency makes challenging the definition of the phenotype associated with copy number variants on this gene.

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E-P09.04

An atypical case of Angelman syndrome who presented with autism spectrum disorder, identified to have uniparental disomy of chromosome 15 by chromosomal microarray

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Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders which are etiologically heterogeneous. Chromosomal microarray (CMA) is now recommended as the first-tier clinical diagnostic test for ASD. We performed CMA in 16 Thai patients with ASD using an Illumina HumanCytoSNP-12 v2.1 array and found one case with uniparental disomy (UPD) of chromosome 15. Methylation-specific PCR (MS-PCR) showed abnormal methylation of the maternal *SNRPN* allele. Haplotype analysis revealed that the patient had received both chromosomes 15 from his father. These results were consistent with Angelman syndrome (AS). However, his clinical features had no clinical significance for classic AS. He had first presented at the pediatric clinic with no speech, poor social interaction skills and repetitive behaviors consistent with ASD based on the DSM-IV criteria at 2 years of age and later confirmed by ADOS at 5 years of age. He had no dysmorphic facies, seizures nor ataxia and was diagnosed as non-syndromic ASD, a diagnosis which was believed until at 10 years of age, his DNA was included for analysis in this current cohort study. Our findings suggest that ASD patients with unknown etiology should be considered for MS-PCR testing for AS where CMA is not available.

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E-P09.05

Multiple heterozygous copy number variants (CNVs) with potential additive effect in patients affected by neurodevelopmental disorders (NDD)

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Emerging evidences suggest that CNVs can likely interact to modulate the individual phenotype in patients affected by neurodevelopmental disorders (NDD). We present two cases with complex NDD phenotype carrying multiple rare CNVs with uncertain significance identified by array-CGH. The first proband is a 2-year-old male child with intellectual disability, mild autism spectrum disorder (ASD), critical episodes, and two arachnoid cysts. He inherited from his healthy mother an intragenic deletion of *CNTNAP2* and from his healthy father a deletion involving *LRRC4C*. Heterozygous *CNTNAP2* deletions seem to cause ASD in combination with variants at other loci. *LRRC4C* deletions were hypothesized to have a modifier effect on NDD. Both *LRRC4C* and *CNTNAP2* encode membrane proteins involved in axon guidance and synaptic adhesion. Half dosage of these two proteins, due to heterozygous deletions, could have additively contributed to neuron dysfunction and phenotype appearance. The second proband is a 9-year-old male child with expressive language disorder, hyperactivity, learning difficulties and movement impairment. His mother presented dyslexia and learning difficulties in her childhood. Mother and son shared a deletion involving *GLRA3*, glycine receptor implicated in synapse formation inhibition through Gephyrin, known to play a role in ASD. The *GLRA3* deletion could be responsible for the phenotypic features shared by mother and son. Two additional *de novo* CNVs could have worsened the patient's phenotype: a deletion of *MIR4465*, negative expression regulator of *PTEN* whose overexpression inhibits axon branching; a duplication encompassing *INPP5A*, phosphatase of Ins (1,4,5)P3 controlling motor coordination by intracellular calcium mobilization, *NKX6-2*, and partially *ADGRA1*.

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E-P09.10

Early onset epileptic encephalopathy and choreoathetosic movements associated with a novel pathogenic variant of *GABRA1* gene

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Introduction: Early onset epileptic encephalopathies (EOEE) are a composite group of severe infantile and childhood onset epilepsies with a heterogenous genetic basis. *GABRA1*-related neurological phenotypes are associated with various forms of epilepsy, ranging from mild generalized epilepsy to severe epileptic encephalopathies. We report herein a 4 years old child with EOEE and severe choreoathetosic movements.

Material and methods: The patient was referred to the clinical genetics department for infantile epilepsy. He originally presented with choreoathetotic movements, subsequent severe epilepsy at five months of age, and severe global developmental delay. No significant dysmorphic features were observed. Extensive metabolic investigations and cerebral MRI were unremarkable. Targeted massively parallel sequencing of a customized panel of 115 epilepsy genes was performed on the patient's DNA.

Results: The molecular genetic study revealed a *de novo* heterozygous variant in *GABRA1* gene: chr5(GRCh37): g.161317987A>G (c.787A>G). This variant has never been described in either pathogenic or polymorphic databases. Three *in silico* algorithms predict this variant as deleterious, and the *de novo* state supports the pathogeneicity. This variant induces the substitution of the amino acid methionine for valine, located on the first transmembrane domain of $GABA_A$-α1 receptor. Other substitutions in this codon have been described as deleterious.

Conclusion: Here we report a patient with a novel *GABRA1* variant inducing EOEE and choreoathetosic movements. This association of symptoms was already reported for *GABRA2* and *GABRG2* genes in the GABA pathway, but had never been linked to a *GABRA1* pathogenic variant.

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E-P09.13
A rare case of juvenile Parkinsonism: Kufor-Rakeb syndrome

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Introduction: Juvenile Parkinsonism affects patients below 21 years-of-age and the background of the disease is very heterogeneous. It can be caused by genetical diseases, degenerative, metabolic, immune-mediated conditions, infections, structural brain abnormalities, toxins and adverse drug reactions. The most common genetical causes includes the alterations of *PARK2*, *PINK1* and *PARK7* genes. Symptomatology also can be very variable including classic Parkinsonian symptoms like rigidity, bradykinesia and postural instability as well as additional symptoms like depression, sleep disturbances, memory loss, constipation and urinary problems.

Materials and Methods: We report a 21-year-old girl with the onset of symptoms at 15 years. Initially symptoms included involuntary laugh and progressive mental decline, later associated with Parkinsonian symptoms.

Results: An apparently homozygous ATP13A2: c.2479G>A variant was identified in exon 22 of the *ATP13A2* gene by Whole Exome Sequencing. The variant is a novel missense variant previously not described in other patients. It is classified as a variant with unknown significance although in silico predictions suggest pathogenicity.

Conclusions: The above mentioned homozygous mutation confirms the diagnosis of Kufor-Rakeb syndrome, which is a rare autosomal recessive juvenile Parkinsonism syndrome. Through our case presentation we would like to shortly review the symptomatology, diagnostics and therapy of juvenile Parkinsonism.

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E-P09.14
An atypical case of *KCNH1*-related epilepsy

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Introduction: Mutations in *KCNH1* [MIM*603305] have been involved in a spectrum of early onset syndromic epilepsies including the well-defined Zimmermann Laband syndrome [MIM#135500] and Temple Baraitser syndrome [MIM#611816]. Thumb abnormalities, defects in the development of the nails, hypertrichosis, gingival hyperplasia, craniofacial dysmorphism and intellectual disability can be considered the hallmarks of *KCNH1*-related syndromic epilepsy. We describe hereby a young boy with *KCNH1*-related epilepsy without typical extra neurologic features.

Case presentation: He is a 4 year-old boy, born to unrelated parents, presenting with an epileptic myoclonic encephalopathy with lingual myoclonus starting in the first 24 h of life. Hypotonia was noted from birth. He evolved subsequently with a major global developmental delay, sensorineural deafness, gastroesophageal reflux and severe constipation. On clinical examination, his limbs are strictly normal, with some elements of craniofacial dysmorphism and slight hypertrichosis. The monogenic epilepsy gene panel revealed a *de novo* variant in *KCNH1*, c.1475C>A, p.(Ala492Asp), interpreted as probably pathogenic.

Discussion: The limb abnormalities, including nail hypo/aplasia and broad thumbs associated with adductus deformities and hypoplasia of distal phalanges are usually

emphasized in the cases reported in the literature. However, we report an individual lacking these classical features.

Conclusion: The association of severe constipation, hypertrichosis and early onset epilepsy in a context of global developmental delay should probably make clinicians consider the diagnosis of *KCNH1*-related epilepsy even in the absence of limb abnormalities.

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E-P09.15

A new KCNQ2 mutation and clinical findings

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Introduction: The KCNQ2 gene encodes the brain voltage-gated potassium channel and is related to benign familial neonatal seizures and neonatal epileptic encephalopathy. 60 and 80% of probands with benign family neonatal epilepsy may be related to the likely pathogenic variants of the KCNQ2 gene. Pathogenic de novo KCNQ2 variants can be detected in some individuals with unexplained afebrile seizures in newborn or early childhood. The aim of this study is to present the changes we detected in a 1-year-old boy who was referred to us for afebril-resistant convulsions.

Methods: After the DNA was isolated from the patient's blood sample, multigenic epilepsy panel analysis including 39 genes associated with epilepsy was performed by next generation sequencing method.

Results: A 1 year-old-boy who was born 3100 gr weight with C/S on his term, was referred us with afebril generalized tonic-clonic seizures started at the age of 4 months. His parents, with no history for epilepsy, made 3rd degree consanguineous marriage. Physical examination revealed no abnormal findings except a 6-month delay in neuromotor development. His cranial MRI, electroencephalogram, abdominal ultrasonography were normal. He has received dual antiepileptic therapy, phenobarbital and levetiracetam. Heterozygote c.459delG (p.Y154fs * 17) likely-pathogenic variant in the KCNQ2 gene, absent in his parents, was detected in his analyse.

Conclusions: The mutation in our patient is not previously reported to be associated with any clinical findings, infantile-onset seizures requiring dual antiepileptic treatment and absence of pathogenic findings in the patient's cranial MRI and EEG shed light on the literature.

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E-P09.18

Rare germline *AKT3* mutation responsible for megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome in two Hungarian siblings

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Introduction: Megalencephaly-polymicrogyria-polydactyly-hydrocephalus (MPPH) syndrome is a developmental brain disorder characterized by enlarged brain size with the cortical malformation bilateral perisylvian polymicrogyria and variable degree of ventriculomegaly. MPPH syndrome is associated with oromotor dysfunction, epilepsy, intellectual disability and postaxial hexadactyly. The molecular diagnosis of this disorder is established the identification of a pathogenic variant in one of *AKT3*, *CCND2* or *PIK3R2* genes. Previously reported *AKT3* gene mutations are associated with various brain involvements and may lead to megalencephaly. Most individuals with MPPH syndrome have a germline pathogenic variant in *AKT3* gene; some have a somatic mosaic pathogenic variant cause hemimegalencephaly, which is similar to MPPH.

Materials and Methods: WES analysis was performed on Illumina HiSeq (2500/4000) instrument using the SureSelect Human All Exon V6 kit for library preparation and the pathogenic variant was validated by Sanger sequencing.

Results: Whole exome sequencing analysis identified a c.1393C>T p.Arg465Trp protein altering pathogenic variant in *AKT3* gene in two affected half siblings but none in the maternal DNA sample therefore it could be a presumably maternal germline mosaicism due to the literature data.

Conclusions: So far 25 cases with 10 different mutations of *AKT3* have been reported. The mutation demonstrated here has been described in 7 patients; however the novelty of our examination is that this is the first occasion, where this type of mutation associated with mosaicism. Project was supported by GINOP—2.3.2-15-2016-00039

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E-P09.19

***CHD2* variants in developmental and epileptic encephalopathies patients (DEEs)**

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The term developmental and epileptic encephalopathies (DEEs) has been recently coined to refer to conditions where the epileptic activity itself contributes to cognitive slowing/regression or to disorders where developmental impairment emerges before the presence of epileptic activity or in the presence of infrequent epileptic activity. Indeed DEEs represent a final common pathway for a broad spectrum of phenotypically and genetically heterogeneous conditions. Next Generation Sequencing is being applied as a routine molecular diagnostic strategy in individuals with DEEs. Here we report the cases of three unrelated individuals in whom, by a targeted NGS approach, we detected three likely/potentially pathogenic *CHD2* variants. The patients were referred for neurodevelopmental phenotypes characterized in all cases by moderate intellectual disability, global psychomotor delay and behavioral disorder/ASD traits, associated with variable epileptic manifestations: absences seizures, generalized tonic-clonic seizures, and/or focal seizures with onset ranging from 9 months to 5 years of age. No photosensitivity was reported. The *CHD2* variants detected were a likely pathogenic novel *de novo* frameshift (c.726_727delAG, p.Ser242fs) located in a functionally relevant phosphorilation site and predicted to result in a premature truncated protein, and two intronic variants both located in splicing regions. These were a *de novo* SNV (c.1719+5G>A) previously reported in literature in 1 affected individual, and a very rare SNV (c.3886-10C>T), found to be paternally inherited. Both intronic variants are currently being further characterized and family segregation investigated and expanded. This report contributes the genotypic and phenotypic characterization of the expanding spectrum of *CHD2* disorders.

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E-P09.21

A first case of OFDVI with c.3545delA and c.7400 +1G>A mutations for CPLANE1 gene

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Introduction: Oral-facial-digital type VI syndrome (OFDVI) is phenotype in the spectrum of Joubert syndrome (JS) and is defined by the presence of the "molar tooth sign (MTS)" with at least one of these findings:(a) tongue hamartoma and/or additional lingual frenula and/or upper lip notch;(b) mesoaxial polydactyly;(c)hypothalamic hamartoma (1).

Case report: A 2 day old boy was referred because of multiple congenital anomalies. Fetal ultrasound of the patients revealed Dandy-Walker malformation. Soon after birth, breathing abnormalities and hypotonia were noticed. Clinical examination showed unilateral mesoaxial polydactyly of hand, complete syndactly in between 4th and 5th fingers of left hand, bilateral preaxial and postaxial polydactyly of feet, tongue hamartoma and lingual frenula. Brain MRI revealed MTS and occipital encephalocele. He was suspected OFDVI. In molecular analysis compound heterozygous for CPLANE1 frameshift mutation c.3545delA; p.N1182I and splice site mutation c.7400 +1G>A was detected.

Results: Considering all reported CPLANE1 mutated patients, over two-thirds showed a pure JS phenotype, while only 24% has OFDVI(2).Mutations detected in our patient have been reported in pure JS previously. However, our case was the first reported OFDVI patient carrying these mutations (c.3545delA/c.7400+1G>A). Genetic modifiers may be responsible from the phenotypic diversity such as in oral-facial and digital features in patients bearing CPLANE1 mutations.

References: 1- Poretti A, Vitiello G, Hennekam RC, Arrigoni F, Bertini E et al., (2012). Delineation and diagnostic criteria of Oral-Facial-Digital Syndrome type VI. *Orphanet J Rare Dis*. 2- Romani M, Mancini F, Micalizzi A, Poretti A et al., (2015). Oral-facial-digital syndrome type VI: is C5orf42 really the major gene? *Hum Genet*.,134(1):123-6.

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E-P09.22

Presence of rare variants in haplotype in *LRRK2* gene as a potential risk factor for endemic parkinsonism

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Introduction: Parkinsonism is relatively common neurodegenerative disorder. A higher prevalence of the disease was observed in the southeastern Moravian region. The study aims to assess the potential genetic risk factors of haplotypes in genes most commonly associated with parkinsonism.

Material and methods: We analyzed 32 patients and 12 controls in *ADH1C*, *EIF4G1*, *FBXO7*, *GBA*, *GIGYF2*, *HTRA2*, *LRRK2*, *MAPT*, *PARK2*, *PARK7*, *PINK1*, *PLA2G6*, *SNCA, UCHL1* and *VPS35* genes. Variants were acquired by Next Generation Sequencing Ion Torrent workflow. Potential candidate haplotypes were assessed using filtering from a manually created matrix. We compared our haplotype frequency with population data from the 1000 Genome Project (1000GP).

Results: We observed shared findings of four intron variants (rs11564187, rs36220738, rs200829235, rs3789329) and one exon variant (rs33995883) in the *LRRK2* gene in six patients. None of which were present in controls. Intron variants occurred almost exclusively together with the exon variant. It can be assumed that there should be variants in haplotype. A comparison with 1000GP data revealed significant differences in haplotype frequencies between the patients and 1000GP "controls". Patients close to consanguinity were excluded by analyzing the HVR1 mitochondrial region and Y-STR loci.

Conclusion: The co-occurrence of five variants in *LRRK2* gene could be a risk factor for the development of parkinsonism.

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E-P09.23

TOR1A dystonia and missense mutations

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DYT-TOR1A dystonia is caused by dominant mutations in the *TOR1A* gene, most frequently a heterozygous in-frame deletion p.302/303delE. The most frequent phenotype is childhood-onset in a limb, spreading to generalized dystonia within a few years. Other in-frame deletions and point mutations in *TOR1A* have been associated with dystonia in a limited number of patients. Here, using gene panel sequencing, we report two new patients with missense mutations in *TOR1A*. We identified a novel likely-pathogenic missense mutation, p.R312G in patient 1, which segregated with mild isolated segmental dystonia in the respective family presenting three affected individuals. Multiple lines of bioinformatic predictions indicate possible deleterious effects on protein function. However, functional analyses and identification of genetic recurrence are warranted to confirm its pathogenicity. Further, we identified a known pathogenic mutation, p.R288Q in patient 2 with adolescent-onset, isolated generalized dystonia, marked axial and little cranial involvement. This mutation was previously reported in a patient with very early childhood-onset lower limb dystonia and severe generalization, who at age 18 had dysphagia, dysarthria, joint contractures, pyramidal signs and cerebellar atrophy. Most reported patients with *TOR1A* missense mutations presented with adult-onset, including the p.R312G index patient in our study. This may indicate that missense mutations have a less profound effect on torsinA function than the common deletion. However, the phenotypic spectrum of *TOR1A* mutations is very broad. The causes of this large phenotypic variation in *TOR1A* mutation carriers still largely remain elusive.

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E-P09.24
Clinical characterisation of hypomyelinating leukodystrophy 14 with founder *UFM1* mutation in Roma population

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Introduction: Hypomyelinating leukodystrophy 14 (HL-14) is recently defined autosomal recessive disorder associated with mutations in *UFM1* gene. We present consistent clinical characteristics of HL-14 patients with c.-273_-271delTCA founder mutation in *UFM1* gene in Slovak Roma population.

Material and Methods: Sanger sequencing of the corresponding region of *UFM1* gene was performed in 8 patients with clinical and/or radiological signs of early-onset leukodystrophy.

Results: The biallelic c.-273_-271delTCA mutation in *UFM1* was confirmed in 8 patients of Roma origin from Slovakia. After typically asymptomatic perinatal period (7 from 8 patients) first clinical symptoms appeared on average at 2.6 month (range 0 to 4). Axial hypotony evolving to acral spasticity, inspiratory stridor and developmental delay were present in all (8/8) patients. Refractory seizures were present in 5/6 patients, in 2 patient the clinical data were incomplete. Secondary microcephaly was documented in 5/5 and severe hypotrophy developed in 6/7 patients. Both hearing and visual impairments were present in 6/6 patients. The tracheotomy was performed in 2/5 patients. The age of death ranged from 3 to 20 months (average 10). Furthermore, we confirmed the presence of c.-273_-271delTCA mutation in three Roma patients of Slovenian origin.

Conclusions: Population isolates are characterized by decreased genetic heterogeneity and occurrence of specific genetic disorders. HL-14 associated with *UFM1* mutation in our group of patients shows a phenotypic uniformity which enables effective targeted genetic testing. Hypothesis arises about ancient origin of the mutation and its probable presence in the Roma population in other European countries.

M. Giertlova: None.

E-P10
Neuromuscular disorders

E-P10.01
Seizures, developmental delay, mild intellectual disability, muscular hypotonia, and optic atrophy: About the genetic diagnosis of patient with a novel ATP1A3 mutation

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Introduction: Different conditions mostly produce overlapping symptoms in rare disorders, instead of characteristically manifesting themselves as repeating collections of stereotypical symptoms. This is the main factor of why some patients remain undiagnosed despite undergoing an exhaustive workup. ATP1A3-related neurologic disorders are extremely rare and represent a clinical continuum of three distinct disease phenotypes, namely rapid-onset dystonia-parkinsonism, alternating hemiplegia of childhood, and CAPOS syndrome.

Materials and Methods: WES is applied for the index case, his healthy brother and healthy non-consanguineous Turkish parents. Evidence from various sources; population databases, computational assessments, PubMed, OMIM, MGI and pathogenicity estimations are gathered. Symptoms of affected individual and family history are reviewed to prioritize variants with the highest degree of symptom match. The genetic diagnosis proposed for the patient is ATP1A3-related neurologic disorder.

Results: Here, we report one additional case of ATP1A3-related neurologic disorder in a fully penetrant classical dominant mode of transmission. We identified a novel mutation in the cytoplasmic domain of ATP1A3.

Conclusions: Molecular testing is the most prominent way that should be added to the routine testing approach for diagnosis of undiagnosed patients with a suspected genetic disorder. We diagnose and report a new pedigree with ATP1A3-related neurologic disorder segregating a *de novo* missense mutation of the *ATP1A3* gene.

C. Saygı: None. **U. Sezerman:** None.

E-P10.02
Sepiapterin reductase deficiency: case report with novel frameshift mutation

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Dopa-responsive dystonia due to sepiapterin reductase deficiency (OMIM#612716) is caused by recessive mutations in the gene encoding sepiapterin reductase (SPR), which plays an important role in the biosynthesis of tetrahydrobiopterin (BH4). One Jordanian patient from a

first cousin parents once-removed is reported. The parents have recognized the symptoms of their daughter at 6 months old with motor developmental delay. The symptoms were progressed after-then to include speech delay, seizure, ataxia, oculomotor apraxia, dysarthia and choreoathetosis. Despite all of these obvious symptoms, the clinicians in Jordan were unable to diagnose the case. In August 2018, the case (10 years old) was presented to the department of biotechnology and genetic engineering at Philadelphia University in Jordan for the purposes of performing whole exome sequencing (WES). Analysis of WES data has revealed novel homozygous frameshift variant in the gene SPR (NM_003124.4):c.40delG;p.Ala15Profs*100 which is heterozygous in the parents and in the healthy siblings. Recommendations were given to the family immediately to start treatments trials. This case illustrates the difficulties of diagnosing sepiapterin reductase deficiency based on clinical symptoms which renders the possibilities of early management and reinforce the importance of running WES for diagnosed cases.

T. Froukh: None.

E-P10.04

A new patient with congenital myasthenic syndrome type 20 due to compound heterozygous missense *SLC5A7* variants revealed using whole exome sequencing

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Congenital myasthenic syndrome (CMS) type 20 (MIM617146) is a rare autosomal recessive neuromuscular disorder caused by missense variants spread across the *SLC5A7* gene. CMS is characterized by severe hypotonia, muscle weakness (causing delayed walking), ptosis, poor sucking and swallowing, hypomimia, oculo-bulbar symptoms and generalized limb fatigability and weakness. The role of *SLC5A7* in CMS type 20 was described in 2016, and since then only 10 patients have been reported. We describe an 8-year-old boy examined since 5 months of age because of episodic apnea due to muscle weakness. He has moderate intellectual disability, autism, ADHD, speech delay, and weakness and fatigability worsening at the end of the day and after physical load. His facial phenotype is not remarkable, except ptosis. Early investigations of several genes associated with CMS known at that time failed to explain the etiology of his condition. Exome sequencing revealed compound heterozygous missense *SLC5A7* variants, paternal c.872T>C, p.(Ile291Thr) and maternal c.1293C>G, p.(Asn431Lys) (NM_021815.4). The maternal variant was absent from all databases. The paternal variant has been described in one patient. Our patient confirms that CMS caused by *SLC5A7* variants can be associated with a wide range of phenotype features, from isolated myasthenia to myasthenia accompanied by significant cognitive impairment and behavioral problems, which separates CMS type 20 from most other types of CMS. Our case illustrates the strength of exome sequencing in deciphering the genetic basis of diseases and contributes to the characterization of genotype and phenotype of this rare disorder. Supported by 17-29423A and 00064203.

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E-P10.05

Novel homozygous *DSTYK* mutation causes late onset spastic paraparesis

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Introduction: Deletions of the last two exons (12&13) of *DSTYK* are known to cause spastic paraplegia (SPG23) in children. Other mutations in the gene have been associated with congenital anomalies of the kidney and the urinary tract.

Materials and Methods: clinical phenotyping, whole exome sequencing, segregation analysis.

Results: Whole exome sequencing of a 51 years old male with progressive spastic paraparesis presenting at age 44 years, displayed a homozygous missense mutation in *DSTYK* (NM_015375.2, c.1840C>T, p.R614W) within an evolutionary conserved sequence of its 7th exon (CADD-Score 35). The frequency of the heterozygous mutation in gnomAD in Europeans was 0.001% while in our in-house database of 362 exomes and in other open-access databases (1000 genomes, ExAC, gnomAD and NHLBI ESP exomes) the frequency was 0%. Additional 100 ethnically-matched DNA samples were tested, none of which harbored the mutation. The proband, child of consanguineous parents of Jewish Moroccan ancestry, has five siblings. Only one of his siblings was homozygous for the mutation—a 43-years old sister, who had been limping for over a year. Neurological exam of both the proband and the sibling pointed to bilateral upper-neuron defect, with specific MRI

findings. Urinary system abnormalities were not identified. The other 4 siblings, not homozygous for the mutation, were asymptomatic.

Conclusions: DSTYK is abundant throughout the central nervous system and is thought to be a mediator of cell death. Our study delineates a genotype-phenotype correlation in *DSTYK* mutations and highlights *DSTYK* mutations in the differential diagnosis of late onset spastic paraparesis.

N. Hadar: None.

E-P10.06

Two families having Huntington's disease with two different clinical presentations

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Introduction: Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder, characterized by choreic movements, dystonia and progressive demans. Since CAG trinucleotide repeats increase during male gametogenesis, the disease occurs earlier than 30 years old, via a transmission through an affected father. Psychiatric symptoms are a significant aspect of HD and highly variable among patients. Their course does not fully correlate with motor and cognitive disease progression.

Materials and Methods: We have two families showing different clinical presentations of HD. In the first family, 30 and 35 years old sisters were initially diagnosed with depression, and then they have started having involuntary movements, impaired coordination, and inability to walk and speak for the last few years. In the second family, 23 and 31 years old brothers have choreic movements, severe demans, seconder epilepsy and bedridden. They had a brother who has died of HD. They all inherited from their affected father.

Results: All patients were diagnosed with HD by using DNA sequencing. Increased (>40) CAG repeats was shown on the exon 1 of the *HTT* gene (on chromosome 4p16.3). The responsible abnormal protein Huntingtin, occurs via CAG repeats, were shown in patients by using Western Blotting.

Conclusion: Overall, although both families have similar number of CAG repeats, our patients in the first family were diagnosed with and treated for depression for many years before clinically and genetically diagnosed with HD, while our patients in the second family were diagnosed with and treated for classical symptoms of HD and seconder epilepsy.

B. Celtikci: None. **F. Kaplan:** None. **R. Sayin:** None.

E-P10.08

A late onset McArdle disease with compound heterogenous novel *PYGM* mutation

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Introduction: Here we present a late onset case of glycogen storage disease type V (McArdle disease; MIM 232600) with compound heterogenous novel mutation of muscle isoform of glycogen phosphorylase (*PYGM*, MIM 608455) mutations. (p.E394fs) Case: 72 years women complain of myalgia in proximal muscled in extremities 2 years before. She showed hyper CK in serum examination and diagnosed as myositis. She visited our hospital for no improvement with the prednisolone and tacrolimus treatment. In neurological examination, she shows mild muscle weakness in proximal region of extremities with myalgia. DTRs are normal and no sensory impairment. Two of her elderly sister and younger brother shows hyper CK emia without any clinical symptoms.

Results: Needle EMG shows myogenic abnormality and muscle biopsy showed mild myogenic change with vacuole. Phosphorylase activity in muscle is absent. Also, in vitro anaerobic lactic acid producing ability is impaired in glycogen, but normal in glucose-1-P. These results suggest this patient suffers glycogen storage disease type V. Gene analysis of *PYGM* revealed that she has compound heterogeneous mutations, one of which is reported (p.P710DEL) and the other is novel (p.E394fs).

Discussion: This is one of the very late onset case of McArdle disease. Late onset McArdle disease is often misdiagnosed as polymyositis or limb-girdle muscular dystrophy and *PYGM* mutations should be looked for in patients with very late-onset myopathy with no previous history of muscle weakness.

T. Furushima: None. **Y. Morita:** None. **Y. Osaki:** None. **Y. Miyamoto:** None. **E. Amano:** None. **T. Horino:** None. **F. Fukuda:** None. **H. Sugie:** None. **I. Nishino:** None. **H. Furuya:** None.

E-P10.09

X-Linked congenital familial hypotonia

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Introduction: Congenital hypotonia is a relatively common diagnosis in the newborn period. Centronuclear myopathy (CNM) is a rare neuromuscular disorder with autosomal-recessive (AR), autosomal-dominant (AD) and X-linked patterns of genetic transmission.

Patient and Methods: First child of a non consanguineous couple. Reduced fetal movements during pregnancy. Born at term with no spontaneous breathing, required endotracheal intubation. Severe hypotonia, hypo mimic face, and cryptorchidism were present. At 1 year of age he carries a tracheostomy tube and a gastrostomy. He can sit but is unable to walk without support. Family history is remarkable for a maternal uncle with neonatal hypotonia, cryptorchidism and ophthalmoparesis; muscle biopsy at 4 months of age was informed as normal, repeated as an adult showed internally located nuclei close to the center of a myofiber. Genetic analysis were performed including a 60K array-CGH, CTG-expansion analysis in DMPK and NGS sequencing of MTM1 gene (X-chromosome). Sanger sequencing was done for variant validation and familiar segregation studies.

Results: No cryptic genomic imbalances were found by aCGH. CTG-expansion analysis in DMPK gene was within normal range. NGS-sequencing revealed the hemyzigous variant c.1181A>C (p.Asp394Ala) in exon 11 of MTM1 gene, not previously described. In silico predictions show potential pathogenicity. Sanger-sequencing was used to establish the maternal origin of the variant, which was also present in his maternal-uncle, while absent in a maternal uncle once removed.

Conclusions: Family history and specific clinical features (undescended testes, ophthalmoparesis) have been determining to make the correct diagnosis and offer adequate genetic counselling

L. Morales-Garófalo: None. **M. Moreno-Igoa:** None. **M. Artigas-Lopez:** None. **A. Bengoa-Alonso:** None. **G. Sierra-Colomina:** None. **A. Ruiz de Sabando:** None. **M. Ramos-Arroyo:** None.

E-P10.10
A further case of *NDUFA2*-related leukodystrophy

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Introduction: Whole Exome Sequencing (WES) has been shown to resolve up to 50% of patients with leukodystrophy. *NDUFA2* variants resulting in complex I deficiency have been recently recognized as responsible of Leigh syndrome or cystic leukoencephalopathy. *NDUFA2* encodes an accessory subunit of complex I.

Case Report: We report a 2-year-old female child born to consanguineous first cousin parents who presented with motor and language regression at 20 months of age, hypotonia, and microcephaly. A brain MRI showed cavitating leukodystrophy with corpus callosum involvement. Trio WES identified the homozygous *NDUFA2* missense variant c.170A>C: p.E57A that was predicted to be damaging (CADD= 31) and was absent in ExAc and GnomAD databases.

Discussion: NDUFA2-related disorder is a mitochondrial disease resulting in leukodystrophy. Only three cases with *NDUFA2* variants have been reported so far in the literature, one presenting with Leigh syndrome and the remaining two with cystic leukoencephalopathy, as in our case. In conclusion, our case confirms that *NDUFA2* variants can be responsible for cavitating leukodystrophy.

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E-P10.11
TRANSLATION OF THE RESEARCH TO DIAGNOSIS THROUGH NEXT GENERATION SEQUENCING

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Introduction: Neuromuscular disorders show a high clinical and genetics heterogeneity. Often, the patients affected by these pathologies show a phenotypic overlap that difficult their diagnosis, especially at early ages. For

this reason, usually these patients haven't got a conclusive molecular diagnosis or their diagnosis has been delayed. In this work we describe our experience in the use of next generation sequencing (NGS) as a key tool for the rare diseases diagnosis. MATERIAL AND METHODS Four different patients with spinal muscular atrophy (SMA) clinical diagnose, in which the involvement of the *SMN1* gene was ruled out as responsible for their pathology. All of them presented a well-defined phenotype using HPO codes evaluated by clinical experts. A clinical exome was performed in all the patients (TruSightOne/TSO,Illumina). In cases with negative results, whole exome sequencing (WES) was performed by Nimblegen SeqCap-EZ-MedExome (Roche). All variants were analysed with Alamut software and confirmed by Sanger. A segregation analyses were carried out to confirm the pathogenicity of the variants identified. RESULTS TSO allowed us to identify c.2362C>T variant in *IGHMBP2* just in one patient. On the other hand, WES was conclusive for the other three patients: i) c.614T>C de novo variant in *BICD2*, ii) two heterozygous variants in *TTN* confirmed by expected familial segregation (c.59626G>A and c.38661_38665del) and iii) c.854C>T variant in *TFG* identified in affected members of the family. CONCLUSIONS—Interdisciplinary collaboration is mandatory to carry out an accurate molecular diagnosis.—Segregation studies are crucial to confirm the pathogenicity of the NGS findings.

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E-P10.12
Incidence of spinal muscular atrophy in Armenia: 2012–2018

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Background: Spinal muscular atrophy (SMA) is the most common genetic cause of infant mortality worldwide. SMA is not studied genetically and epidemiologically in Armenia.

Objective: To describe epidemiological characteristics of patients with SMA and to study incidence of genetically confirmed SMA in Armenia.

Methods: This is an observational retrospective study based on analysis of registry of CMG in Armenia from 2012 to 2018. The study population included all patients with clinical suspicion of SMA, who were referred for genetic testing.

Results: In the sample of 111 patients 99 were referred for postnatal and 12 for prenatal testing during the period of 2012–2018. In 99 cases among 37 (37,4%) SMA was confirmed as having homozygous deletion of SMN1 exon 7. Prenatal testing was carried out in 12 pregnant women and among 2 the disease was confirmed and the pregnancy was terminated. Among postnatal SMA patients 59,5% were children within 6 months of age (SMA 1), 27% were in the range of 7–12 months (SMA 2) and 10.8% were children between 2–15 years of age (SMA 3). In Armenia, there were 280,600 live births over the period of 2012–2018. The calculated incidence of SMA in Armenia is approximately 13,2 in 100,000 newborns which is comparable with incidence observed in Eastern European countries.

Conclusion: This study shows epidemiological picture of SMA in Armenia and suggests that practitioners should refer early for genetic testing. This will facilitate management of SMA and will help to identify patients potentially ready for new therapies.

K. Hovhannesyan: None.

E-P10.13
A second case of spinal muscular atrophy with congenital bone fractures-2 due to rare loss of function mutation in *ASCC1* gene in Bulgarian patient of Roma origin

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Introduction: Spinal muscular atrophy with congenital bone fractures-2 (SMABF2) is a severe autosomal recessive condition presenting with arthrogryposis multiplex congenita, multiple prenatal fractures of the long bones, and generalized muscle atrophy caused by mutations in the *ASCC1* (Activating Signal Cointegrator 1 Complex, subunit 1) gene on chromosome 10q22.

Materials and methods: Herein we describe a female newborn of consanguineous parents of Roma origin with severe muscle hypotonia (reduced spontaneous movements, absent reflexes, neonatal respiratory distress syndrome) and congenital long bone fractures (initial diagnosis of

osteogenesis imperfecta). The patient deceased 2 months after birth.

Results: We have previously reported a case of a newborn boy with SMABF2 from a consanguineous family of Roma origin who deceased 12 days after birth. A loss of function donor splice-site mutation c.710+1G>A in *ASCC1* gene was detected by NGS and result was validated by Sanger sequencing and segregation analysis in the affected family. Based on the similar clinical data observed in the current patient we started the diagnostic process with DNA analysis for the same disease causing mutation *ASCC1:* c.710+1G>A. The Sanger sequencing testing confirmed the diagnosis of SMABF2 due to the mutation in homozygous state.

Conclusions: The results in our two patients disclose a presumptive high carrier frequency of the mutation c.710 +1G>A in *ASCC1* gene in Bulgarian Roma population due to founder effect. The diagnostic difficulties are due to the rarity and the fatal outcome of the condition. Implementation of population based genetic screening is an option to be discussed.

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E-P10.14
Detection of a homozygous deletion of *ASCC1* gene by NGS in a patient with arthrogryposis and generalized hypotonia

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ASCC1 encodes a subunit of the ASC-1 complex, which by binding with nuclear receptors can affect transcription, both as repressor and coactivator. It also participates in pre-mRNA processing and regulation of splicing and has been associated with neuro- and myogenesis. Mutations in ASCC1 are correlated with spinal muscular atrophy with congenital bone fractures type 2 (SMABF2, OMIM #616867), which manifests by hypotonia, muscle weakness caused by degeneration of motor neurons, arthrogryposis and bone fractures. We present a case of 2 year old girl with homozygous deletion of 2 exons in ASSC1 gene, detected by next generation sequencing (NGS). The child was born in 34 week of pregnancy complicated by hydrops fetalis and cardiomegaly. Massive soft tissue swelling, dysmorphia, hand contractures, talipes and generalized hypotonia, spontaneous fracture of left femur were present after birth. Chromosomal aberrations and SMN1 deletion were excluded. Metabolic tests results were normal. NGS assay didn't identify known pathogenic variants that could be correlated to existent symptoms. Homozygous deletion of 2 exons in ASSC1 gene was detected in two applied tools for CNV evaluation and has been validated by other molecular method. The variant has not been previously described but due to its molecular character and localization has been evaluated as likely pathogenic. As only few cases of SMABF2 have been reported, none caused by deletion, our study makes a contribution to expanding its molecular and clinical spectrum. As well it proves that NGS method can be successfully employed to detect CNV in rare neuromuscular diseases.

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E-P11
Multiple malformation/anomalies syndromes

E-P11.01
16p11.2 recurrent rearrangements: clinical presentation and molecular description of array CGH results

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Introduction: Deletion 16p11.2 syndrome is a contiguous-gene disorder associated with characteristic recognizable pattern of dysmorphic features, congenital anomalies, macrocephaly, developmental delay (DD), autism, hypotonia and obesity, while duplication 16p11.2 presents overlapped features with the deletion but with more grossly dysmorphic features, microcephaly and underweight. Materials and Methods DNA was extracted from peripheral blood from a total of 1200 pediatric cases with ID, DD, with or without multiple congenital anomalies. Chromosomal Microarray Analysis (CMA) was done using the 4X180K G3 CGH+SNP microarray platform (Agilent Technologies, Santa Clara, CA, USA). Results 9 deletions and 2 duplications of the 16p11.2 genomic region were identified accounting for 0.9% of all samples submitted for array CGH analysis. Five deletions included proximal 16p11.2 region (BP4-BP5) while four included the distal 16p11.2 region (BP2-BP3). The two duplications were both distal (BP2-BP3). 4/9 patients with the deletion, presented flat midface, broad forehead, hypotonia, macrocephaly, obesity and developmental delay, mainly speech delay, 1/9 microcephaly, 3/9 presented congenital anomalies, while none of them had seizures. One patient had inherited the deletion

from a phenotypically healthy father. The two patients with the duplication had additional pathogenetic aberrations (Del 10p.3/Del 9p24.3 and Del 1p36 respectively) resulting in more severe phenotype, with multiple congenital anomalies, developmental delay and seizures.

Conclusions: Our clinical and molecular findings for the deletion supports the so far distinct phenotypic consequences of the syndrome, however the duplication seems to be more severe for certain phenotypic features (i.e. speech delay, dysmorphic features), which may be due to the additional imbalances.

K. Kosma: None. **A.K. Mitrakos:** None. **M. Poulou:** None. **M. Tzetis:** None.

E-P11.02
A further case with 16q12.1q21 deletion and refinement of critical region

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Introduction: Interstitial centromeric deletions of 16q chromosome including 16q12.1q21 region are rare with only 3 cases reported to date. Main clinical features include dysmorphisms, short stature, microcephaly, eye abnormalities (myopia and strabismus), epilepsy, development delay, intellectual disability, and autism spectrum disorder (ASD).

Case report: We report a 12 year and 4 month-old boy with dysmorphic facial features including depressed nasal root, long philtrum, cupid's bow shaped upper lip, short stature, microcephaly, developmental delay, severe myopia, strabismus, and aggressive behaviours but not ASD. A minimal region of overlap spanning 1.7 Mb on chromosome 16 including *GNAO1* and a cluster of metallothionein genes was found among the case described here and those previously reported in the literature.

Conclusions: Our case suggest a more precise definition of the contribution of genes in the 16q12.1q21 region to the phenotype of affected individuals carrying deletions of this region. Interestingly, this minimal deleted region of overlap includes *GNAO1* and genes encoding metallothioneins which have been implicated in various diseases of the central nervous system.

D. Apuzzo: None.

E-P11.03
Del 22q11 phenotypic delineation from a group of patients. Differences between our patients clinical findings and the literature

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Introduction: The 22q11.2 deletion syndrome is considered the most frequent chromosomal microdeletion. The incidence and prevalence is quite variable. Some studies estimate the range of 1: 3000 to 1: 6000 live births, but this depends on the diagnostic clinical suspicion to carried out the chromosomal and FISH studies. Material and methods. 22 patients were recruited in the Department of Genetics. The chromosome analysis was performed with the standard GTG bands technique. FISH studies were carried out using the elimination probe of the VCFS region TUPLE 1 (Visys) that covers the entire TUPLE1 gene. A total of 20 metaphases and 200 nucleous from each patient were examined.

Results: Of the 22 patients, 12 were men and 10 women. The average age was 13 years. Only 1 patient had IUGR and 8 had short stature. They also frequently had submucosal cleft palate (13), facial dysmorphism (22), conductive type hearing loss (8) and absence of cardiovascular disease in 11 patients. Sixteen of the 22 patients had some degree of mental retardation.

Other findings found in our patients, mentioned as less frequent in the literature, were: epilepsy (11), hypotonia (11), malformations of the extremities (18).

Conclusions: It is very important to pay attention to the characteristics that are not very frequent and also to those mentioned as frequent that patients might not have. We think there is a bias due to the patients attended in our institution. For this reason we find absence and / or less serious cardiovascular malformations.

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E-P11.04
Genotype-phenotype analysis in 22q11.2 deletion/duplication groups

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Introduction: Genomic rearrangements of 22q11.2 region are common and demonstrate variable phenotype of microdeletions/microduplications. We studied genotype-phenotype correlations in 22q11.2 deletion (del22q11.2)/duplication (dup22q11.2) prenatal and postnatal cohorts focusing on clinical and molecular findings and compared results with previously described in the literature.

Materials and Methods: A retrospective study enrolled Lithuanian patients with del22q11.2/dup22q11.2 identified by SNP-CGH and FISH analysis from 2011 to 2018. Data of 22 patients with del22q11.2 and 6 with dup22q11.2 was included.

Results: In 21 patients identified by SNP-CGH the size of deletions ranges from 2.26 to 2.62 Mb, duplications—1.5–4.14 Mb. All deletions and 5/6 duplications encompass *TBX1* gene. At the time of diagnosis patients in postnatal del22q11.2 group exhibited developmental delay/intellectual disability (13/18), congenital heart defects (12/18), and recurrent infections (7/18); most frequent phenotypic features were small, low-set, deformed ears (13/18), small mouth (8/18), minor anomalies of hand and feet (6/18 each). The postnatal dup22q11.2 group demonstrated developmental delay/intellectual disability (4/5), hypotonia (4/5), impaired fluency of speech (3/5); most common phenotypic features were high forehead (3/5), synophris (2/5), minor anomalies of hands (2/5). In the prenatally diagnosed cases 3/4 fetuses with del22q11.2 presented with cardiac anomalies and facial dysmorphism, while fetus with dup22q11.2 showed no distinguishable features.

Conclusion: We observed high prevalence of developmental delay/intellectual disability in del22q11.2/dup22q11.2 cohorts. *TBX1* is known to be one of the causes of intellectual disability in these syndromes. However, specific del22q11.2 features were not characteristic to dup22q11.2 patients and distinct phenotype associated with dup22q11.2 remains challenging to establish.

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E-P11.06
2q37 microdeletion, not including HDAC4, in a family with intellectual disability, microcephaly, facial dysmorphisms and congenital heart defects

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Introduction: 2q37 microdeletion syndrome is one of the most common subtelomeric deletions, characterized by intellectual disability, facial dysmorphisms, obesity and brachydactyly. Mutations in *HDAC4*, a gene coding a histone deacetylase mapped to 2q37.3, were associated with intellectual disability, obesity and brachydactyly. The gene *HDAC4*, has been suggested as the main gene responsible for the 2q37 microdeletion syndrome phenotype.

Materials and Methods: We studied a mother and two sons with short stature, mild intellectual disability, facial dysmorphisms, microcephaly and congenital heart defects. No limb abnormalities were identified. Chromosome microarray analysis was performed using Cytoscan 750K (Affymetrix).

Results: Chromosome microarray analysis revealed a 2,2Mb terminal deletion at 2q37.3 (arr[hg19]2q37.3 (240,541,780–242,782,256)x1). The proximal breakpoint is located immediately downstream of *HDAC4*. All three individuals carried the deletion.

Conclusion: The deletion reported in this family includes all genes in the critical region for 2q37 microdeletion syndrome but not *HDAC4*. Though the role of *HDAC4* in brachydactyly and obesity is clear, our results show that deletion of the remaining genes is also responsible for a syndromic phenotype and thus significantly contribute to the 2q37 microdeletion syndrome.

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E-P11.08
Aarskog-Scott syndrome: follow-up of 2 related patients

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Aarskog-Scott syndrome (AAS) is a rare genetic disorder that primarily affects males while women present attenuated phenotypic features. AAS is characterized by growth deficiency, dysmorphic features, digital and genital anomalies. Mutations in the FGD1 gene are the known cause of AAS (Xq11.22).

We describe a family with AAS (2 cousins and their mothers) diagnosed in Iasi Medical Genetics Centre, to illustrate the relevance of clinical features for the diagnosis, differences between individuals and their long-time evolution. The cases were confirmed using DNA sequencing that identified a deletion in the FGD1 gene.

Case 1 (male, 12 years): growth deficiency (recovered under GH replacement therapy), microcephaly, typical dysmorphic face, myopic astigmatism, nasal voice, short neck, mild pectus excavatum, brachydactyly with interdigital webbing, bilateral camptodactyly of the 4th and 5th finger, short/wide feet with mild brachydactyly, shawl scrotum, bilateral cryptorchidism (surgically corrected), minor heart defects, enuresis, delayed bone age, mild intellectual disability.

Case 2 (female, 8 years old): growth deficiency, microcephaly, dysmorphic face, right eye divergent strabismus, nasal voice, bilateral single transverse palmar crease, brachydactyly, mild interdigital webbing, bilateral camptodactyly of the 4th and 5th finger, mild bilateral clubfoot in infancy, functional systolic murmur, mild intellectual disability and ADHD.

Both mothers have a very mild phenotype.

The long-term follow-up of our patients revealed a changing of their clinical particularities that will be illustrated more with pictures in the poster.

In conclusion, we present a familial case of AAS with particular features, to discuss long-term evolution and multidisciplinary approach.

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E-P11.09

Copy number variation analysis in patients with anorectal malformation and cleft lip and palate in order to determine the frequency of 22q11.2 CNVs and identify rare disease-causing CNVs

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Introduction: Microdeletions and -duplications of 22q11.2 are common aberrations causing various congenital malformations. 22q11.2 deletions are rare (0.3%) in patients with cleft lip and palate (CLP) and also rarely found in patients with anorectal malformation (ARM). In order to determine the frequency of 22q11.2 aberrations and identify novel aberrations in families with the combination of ARM and CLP, we performed a systematic genome-wide analysis of copy number variations (CNVs).

Methods: In ten patients with ARM and CLP array-based molecular karyotyping was performed using the Illumina GSA-1.0. CNVs were calculated from raw intensity data. Common CNVs were excluded by filtering against 4168 healthy controls using PLINK 1.07. Remaining CNVs were evaluated manually using GenomeStudio2.0 genotyping module, filtered for gene content (UCSC) and for their presence in the Database of Genetic Variants. Every patient has been inspected visually at the 22q11.2 locus using GenomeStudio.

Results: In one patient with ARM, CLP and ventricular septal defect a 3Mb deletion of 22q11.2 was identified (chr22:19,241,624–22,323,048 (hg19)). In the genome-wide analysis we detected four novel possibly disease-causing CNVs in three patients. These findings are currently studied for validation, familial segregation and content of possible candidate genes.

Conclusions: Our investigation of a small cohort of families with the combination of ARM and CLP suggests a high prevalence of 22q11.2 aberrations. However, sample size is limited and the analysis of larger cohorts is necessary to obtain reliable figures. The evaluation of four novel CNVs containing possible candidate genes is currently ongoing. **Grant** BONFOR O-120.0001 and O-2018-4-01

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E-P11.10

Deletion and duplication syndromes: Four cases of atypical chromosomal rearrangements

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Objectives: Partial chromosomal deletions and duplications —copy number variations—are a major contribution to the genome variability among individuals and can be either pathogenic or without clinical consequences.

Methods: We present clinical, cytogenetic, molecular and genotype-phenotype analysis of four cases representing atypical forms of syndromes. Microarray-based comparative genomic hybridization (array CGH; Agilent 8x60K) was used for characterization of atypical forms of syndromes. All findings were confirmed by FISH and we also investigated the parental origin of rearrangements.

Results: In the first case, we report diagnosis of an atypical form of Williams-Beuren syndrome due to a *de novo* 3,7 Mb 7q11.23 deletion detected in a one-year-old

girl with psychomotor delay and facial dysmorphism. The second case presents a 4,5 Mb duplication at 7p12.2p11.2 in a fetus with omphalocele. Duplications of 7p11.2-p13 have been described in some cases of Silver-Russell syndrome. Array CGH analysis in the parents was performed only in the mother and showed a 1,2 Mb distal 22q11.2 duplication. In the third case, we found a *de novo* 15 Mb duplication at 14q32.12q32.33 related to 14q distal duplication syndrome in a fetus with cerebral ventricular dilatation and karyotype*:* 46,XX,der(5)(5pter→5q35.3::22q13.1→22qter),der(22)(22pter→22q13.1::14q32.12→14qter). A girl with short stature, hand and foot abnormalities and congenital duodenal atresia represents the last case. In this case, we identified *de novo* 2,9 Mb and 2,0 Mb deletions associated with 13q deletion syndrome and also *de novo* t(2;13).

Conclusions: Some deletion and duplication syndromes (including atypical forms) continue to be a huge challenge when it comes to clinical interpretation.

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E-P11.11
A deletion and a duplication in the same chromosome by array CGH

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Introduction: Partial duplications and deletions on the same chromosome are very rare and usually a sporadic event. They could be due to a parental paracentric inversion of that chromosome, non-allelic homologous recombination (NAHR) or to an abnormal cross-linking during meiosis. Also, the phenotypic expression of both aneuploidies could be highly variable and the etiological mechanism is complex.

Methods: Here we present a 6-years-old girl with growth delay and autism spectrum disorder (ADS). Normal cytogenetic study was conducted two years ago. We performed array CGH in peripheral blood. Cytogenetic studies were carried out in the parents after the child results.

Results: The patient shows a deletion and a contiguous duplication in chromosome 7, at the region adjacent to the *ELN* gene without affecting it:

arr[GRCh37] 7q11.21q11.23(62030284_72401145)x1, 7q11.23q21.11(75061927_83488169)x3.

Cytogenetic studies in the parents showed a paracentric inversion in the long arm of chromosome 7: 46,XX,inv(7)(q11.21q21.1) in the mother, whilst in the father no structural or numerical chromosome abnormalities were observed.

Conclusions: Array CGH has allowed identifying the aetiology of the patient: 7q11.21q11.23 interstitial deletion and 7q11.23q21.11 interstitial amplification. The existence of paracentric inversion in the mother completely changed the risk of recurrence for the parents in case of future pregnancies, getting personalized genetic counselling.

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E-P11.12
The first report of an apparently balanced translocation t(1;2)(q42.1p25.3) responsible for abnormal offspring

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Introduction: we report a female patient 5 years old of Hungarian and Syrian origin that was referred to the Department of Medical Genetics due to macrocephaly, prominent forehead, dysmorphic facial features, congenital anomalies, severe developmental delay, intellectual disability, stereotypic movements, absent speech, poor eye contact and disability of walking alone. She was born at 39 weeks gestation, by normal uneventful delivery, after a pregnancy without medical care.

Methods: cytogenetic analysis was performed on GTG-banded metaphases at a resolution of 500 bands approximately.

Results: cytogenetic analysis revealed that the patient was a carrier of a derivative chromosome 2, characterized from the addition of supplement material of unknown origin in the area p25.3, der(2)(?::p25.3). Parental karyotype indicated that the mother carried an apparently balanced translocation: 46,XX,t(1;2)(q42.1p25.3).

Conclusion: this is the first report of an apparently balanced translocation t(1;2)(q42.1p25.3) contributing to the etiology of abnormal phenotype. Timely diagnosis of parental balanced chromosomal rearrangements can reduce the risk of abnormal offspring.

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E-P11.13

Beckwith-Wiedemann Syndrome: approach to increase rate

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Introduction: Within the differential diagnosis of patients who present hemihypertrophy/hemyhyperplasia, macrosomia, macroglossia and neonatal hypoglycemia, one of the entities that should be discarded, due to the implications in the follow-up that may subsequently have, is the Beckwith-Wiedemann syndrome (BWS).

Material and Methods: We retrospectively analyzed all patients who undergone MLPA methylation 15q13 (Beckwith-Wiedemann) and CDKN1C sequencing during the last 12 years in our Department of Clinical Genetics. We analysed results obtained according to the diagnostic suspicion, clinical criteria that fulfilled, as well as the tissue that was analyzed.

Results: In total we performed 69 MLPA of methylation for BWS, of these, 18 cases presented clinical suspicion of BWS and in 31 cases was hemihypertrophy. Of all these MLPA studies,12 were pathological: 6 IC2 hypomethylation, 1 hypermethylation IC1, 5 uniparental disomy mosaic. 4 of them were negative in peripheral blood but positive in a sample taken from affected tissue. Two patients had mutations in CDKN1C.

Conclusions: It is important to cover all aspects of samples for analysis in search of a possible mosaic and perform the study in an affected tissue (not only to keep a peripheral blood study), given that the rate of diagnosis increase.

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E-P11.15

Clinical and molecular characteristics of Russian patients with CACP syndrome

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Introduction: CACP syndrome (Camptodactily—Arthropathy—Coxa Vara—Pericarditis) is a rare autosomal-recessive condition. It is caused by mutations in PRG4 gene, which affect viscosity of synovial and pericardial fluid due to impaired lubricin production. CACP syndrome patients are often misdiagnosed as having juvenile idiopathic arthritis and receive improper immunosuppressive therapy. The disease leads to joint contractures and early-onset arthrosis. While the condition has been reported in many ethnically diverse populations, the data on its prevalence in Russia are scarce. Here we describe clinical and molecular characteristics of Russian patients with suspected CACP syndrome.

Materials and methods: 11 patients from 8 unrelated families with clinical signs of CACP syndrome were subjected to Sanger sequencing of PRG4 gene.

Results: Pathogenic mutations affecting both alleles were found in 4 of 11 children (36%); 2 variants have not been reported previously. Another 4 patients carried heterozygous PRG4 alteration. The remaining 3 patients had had normal sequence of PRG4 gene. The molecular analysis was compromised by the presence of multiple repeats in a part of exon 6.

Conclusions: This report extends current data on the spectrum of molecular alterations related to CACP syndrome. This work has been supported by the Russian Foundation for Basic Research (RFBR grant 17-29-06069).

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E-P11.16

An apparently balanced complex chromosome rearrangement involving eight breaks and five chromosomes in a healthy female and segregation/recombination to her affected son

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Introduction: Complex chromosome rearrangements (CCRs) are structural alterations involving 3 or more chromosomes. We report on a healthy female carrying a balanced CCRs involving at least 8 breaks and 5 chromosomes; the affected son inherited 3 of the derivatives and one recombinant chromosome.

Patient and Methods: chromosome microarray analysis (CMA) was performed using the Illumina 850K platform. FISH was performed using whole-chromosome libraries and selected BACs.

The propositus is a 2 months old male, referred for evaluation due to pre and postnatal growth restriction, atrial septum defect, neurodevelopmental delay and minor morphological anomalies. His mother had two miscarriages and one healthy daughter.

Results: CMA on the affected proband revealed gains of 20,3 Mb and 11,8 Mb segments from 1q and 4p, respectively (arr[GRCh37] 1q41q43(219,446,253–239,755,240)x3;4p15.2p14(25,803,830–37,653,656)x3).G-banded karyotype from the mother revealed 46,XX,t(1;14;18;4)(q?41;q?24;q?21;p?15). FISH in the mother's metaphase using chromosomes 14 and 18 libraries confirmed a 14;18 translocation. FISH using BAC probes showed that the duplicated 1q41q43 and 4p15.2p14 segments were inserted on 1p and 13q, respectively. The rearrangement in the mother can be described as:46,XX,ins(1p?32q41q43),ins(13;4)(13q?21;4p15.2p14),t(14,18)(?q24,?q21) The son inherited the balanced t(14,18), two normal chromosomes 4, the chromosome 13 with the 4p insertion, and a recombinant between the rearranged and the normal chromosomes 1 from the mother, resulting in extra material from 1q.

Conclusion: CCR population frequency may be underestimated, since some CCRs may not elicit a phenotypic effect. The case presented shows a balanced CCRs uncovered in a healthy female after investigation of an affected offspring carrying an unbalanced karyotype.

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E-P11.17
Identification of two novel *BCOR* mutations with *de novo* occurrence

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Introduction: Congenital cataract (CC) is defined as an opacification of the lens present at birth or appearing shortly thereafter. The etiology is diverse and includes genetic, infectious and nutrition causes. A considerable amount of isolated CC is inherited as a monogenic trait with mutations in more than 330 genes implicated to date. As a part of our ongoing study, we have investigated two probands with CC.

Materials and Methods: We performed exome sequencing using Agilent SureSelect XT_V5_PostCap. Reads were aligned to the GRCh37/hg19 human reference sequence with Novoalign. Annotated variants with a minor allele frequency ≤ 0.005 as per gnomAD in genes implicated in the pathogenesis of CC were prioritized for further analysis. Sanger sequencing was used to confirm the presence of the detected mutations and for segregation analysis.

Results: Two novel mutations in the *BCOR* gene c.3914dupA; p.(Gln1306Alafs*20). c.2382delG; p.(Lys795Argfs*12) were identified. In both instances the parents did not carry the mutation indicating a *de novo* occurrence. *BCOR* mutations cause an x-linked oculofaciocardiodental syndrome manifesting as a variable combination of CC, facial dysmorphism, cardiac, dental and toe anomalies. Both probands denied any other symptoms, however marked teeth anomalies could be readily noted.

Conclusion: A considerable number of sporadic patients with CC harbour *de novo* mutations transmitted as an x-linked or autosomal dominant trait. Exome sequencing can have a significant impact as establishing molecular diagnosis of severe syndromic disease has further implications for clinical management and preconception counselling.

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E-P11.18
Prenatal diagnosis of Costello Syndrome by exome sequencing

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Introduction: We present a prenatal case where clinical exome sequencing allowed for the diagnosis of Costello syndrome (CS), a rare, multiple anomaly congenital syndrome.

Materials and Methods: Second trimester ultrasound investigation revealed a fetus with bilateral camptodactyly, hydramnios and relative macrocephaly. Amniocentesis was performed at 22 weeks gestation age and chromosomal abnormalities were ruled out by conventional and molecular karyotyping. Clinical exome sequencing was performed on the DNA extracted from amniotic fluid, using Sophia Genetics' Clinical Exome Solution v2, which includes 4,493 clinically important genes. Following preparations, according to the manufacturer's protocol, DNA libraries were sequenced on an Illumina NextSeq-500 genetic analyser. Data processing, variant calling and pre-classification were conducted by SOPHiA DDM® bioinformatics pipelines.

Results: A pathogenic variant, c.34G>A (p.Gly12Ser) (rs104894229/NM_001130442) was identified in the *HRAS* gene, previously reported in CS patients. Following clinical genetic counselling, the pregnancy was terminated. Post mortem pathology findings were consistent with the syndrome.

Discussion: Recognition of CS, a rare congenital anomaly, in utero is very important because of the neonatal risk of cardiac mortality and morbidity. Ultrasound findings are unspecific and appear late in pregnancy making genetic investigations challenging. Clinical exome sequencing deciphered the genetic abnormality thus allowing timely healthcare decisions. This is a paradigm of exome sequencing's contribution to the field of prenatal diagnosis.

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E-P11.19

A girl with an inherited central deletion 22q11.21 resulting in DiGeorge syndrome without typical congenital anomalies

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Introduction: Central deletions 22q11.21 are quite rare, only limited numbers of individuals have been reported. Deletions are nested at the distal end of the most common ~3 Mb region resulting in DiGeorge (DGS)/velocardiofacial syndrome but do not contain the DGS critical genes *HIRA* or *TBX1*. These "atypical" deletions exhibit a highly variable clinical phenotype. They are challenging to recognize, share many features with the "typical" DGS and show high level of inheritance.

Case presentation: Here we present a 9-year old girl referred to genetic counselling for inflammatory bowel disease and short stature. Array CGH analysis was performed and identified a deletion in 22q11.21 region located between low copy repeats blocks LCR22-B and LCR22-D, spanning 35 genes including *CRKL*. Neither *TBX1* nor *HIRA* were deleted. This same microdeletion was detected in apparently phenotypically normal father who has a history of recurrent infections and basaliomas.

Conclusions: Patients with DGS may not be severely affected. Sometimes it is hard to recognize their phenotype. Clinical features of DGS are in general milder in related family members than in *de novo* cases. Affected parents can demonstrate extremely mild phenotype or no features at all. Due to mild demonstration of clinical features numbers of affected individuals remain undiagnosed.

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E-P11.20

The potential of array- and NGS-based diagnostics: a *FOXP1* mutation, a 16p11.2 microdeletion and 45,X/46,XX mosaicism explaining the phenotype, and a pre-CLL as an incidental finding

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Introduction: About 6% of patients with intellectual disability (ID) have more than one genetic condition, but finding more than two genetic aberrations is rare. We present a 55-year old female with severe ID, no speech,

short stature and macrocephaly, and four important findings upon routine genetic laboratory work-up.

Results: Firstly, a SNP-array revealed a classical ~0.6 Mb 16p11.2 microdeletion. In addition, copy-number ratio suggested >six mosaic chromosomal aberrations, including loss of TP53 and 13q14.3, consistent with chronic lymphatic leukemia (CLL). Secondly, a routine karyotype was done, and a single likely CLL precursor cell metaphase was found in addition to 45,X[4]/46,XX[31] mosaicism. In a follow-up unstimulated 24 h blood culture, interphase-FISH confirmed TP53 deletion in 44% of cells. Flow cytometry confirmed circulating monoclonal B-cells, but due to low number (<5 x 10^9/L) and the absence of systemic features of lymphoproliferative disorder, this was classified as monoclonal B-cell lymphocytosis (MBL) and not CLL. Finally, an NGS-based 1165-gene panel revealed heterozygosity for a recurrent pathogenic *FOXP1*-mutation: NM_001244808.1 c.1538G>A p.(Arg513His).

Conclusion: *FOXP1* mutations correlate with severe ID without speech, and 16p11.2 microdeletions with ID, obesity and macrocephaly. This combination explains her phenotypic traits except short stature, possibly caused by 45,X/46,XX mosaicism. The most unexpected finding was her monoclonal B-cell expansion, which may develop into CLL. While MBL and clonal aberrations are not uncommon in the healthy population (reported in ~5% in her age group), an incidental finding of MBL on SNP-array is very rare, and clinical follow up is recommended.

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E-P11.21
Description of a severe form of Frank-ter Haar syndrome and literature review

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Frank-ter Haar syndrome (FTHS) is a rare autosomal recessive syndrome resulting from mutations in the *SH3PXD2B* gene, involved in the formation and function of podosomes. FTHS is characterized by facial dysmorphism, megalocornea, inconsistent glaucoma, skeletal and cardiac anomalies, and developmental delay. To date, 36 patients have been reported in the literature, including 17 with identified mutations, only. Among the latter, 6 have congenital glaucoma and 4 died in early childhood from cardiac failure or unknown cause. We present a review of the 17 patients previously described in the literature and report on the first girl born to consanguineous parents, with prenatal hypotrophy, hypotonia, congenital glaucoma, caudal appendix, camptodactyly and craniofacial features suggestive of FHTS. The patient also had ventricular septal defect, dorsolumbar scoliosis, and thin corpus callosum. Clinical evolution resulted in buphtalmos worsening, coarsening of the facial features and respiratory failure with ventilatory dependence leading to death at 5 months of age. Diagnosis of FTHS was confirmed by the identification of a deleterious homozygous mutation in *SH3PXD2B* (c.969delG), already described in the literature. This is the first description of very severe phenotype with non-infectious and lethal respiratory impairment in FTHS, confirming the clinical heterogeneity of this rare condition. We were not able to determine a genotype-phenotype correlation since very few patients are described in the literature. In addition, 2 out of the 3 patients carrying this mutation had a favorable clinical course. However, more cases are needed in order to better characterize the phenotype and establish genotype-phenotype correlation.

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E-P11.23
The Clinical features of individuals of Hypotonia, ataxia, and delayed development syndrome (HADDS) with recurrent *EBF3* mutations

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Hypotonia, ataxia, and delayed development syndrome (HADDS) is a neurodevelopmental syndrome caused by heterozygous mutation in the EBF3 gene (MIM; 607407) on chromosome 10q26. The *EBF3* gene encodes one of a family of highly homologous transcription factors. EBF3 is itself a downstream transcriptional target of ARX (MIM; 300382), and thought to be transcriptionally repressed by ARX [Chao et al., 2017]. HADDS is characterized by congenital hypotonia, delayed psychomotor development, variable intellectual disability with speech delay, variable dysmorphic facial features, and ataxia, often associated with cerebellar hypoplasia [Sleven et al., 2017]. Some patients have urogenital abnormalities. We reported the clinical details of two individuals with HADDS caused by recurrent mutations in *EBF3*. Individual 1 is a 5 years old Japanese girl with nonconsanguineous parents. She showed hypotonia, developmental delay, microcephaly, cerebellar hypoplasia, strabismus, neurogenic bladder, and vesicoureteral reflux. We identified a *de novo* missense mutation, c.471C> A: p. (His157Gln) by trio whole exome sequence. The mutation located at the highly conserved DNA binding domain. Individual 2 is a 2 years old girl with nonconsanguineous parents. She also showed hypotonia, developmental delay, microcephaly, cerebellar hypoplasia, strabismus, neurogenic bladder, vesicoureteral reflux, and clubfoot. We identified a recurrent missense mutation, c.625C > T: p.(Arg209Trp) by Sanger sequence. Our report further supports the notion that *EBF3* mutations cause phenotype of cerebellar hypoplasia and urogenital abnormalities in patients with HADDS.

E. Nishi: None. **T. Uehara:** None. **K. Kosaki:** None. **N. Okamoto:** None.

E-P11.24
Hirschsprung disease caused by a de novo interstitial deletion of 13q21.33-q31.1

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We report on a ten months old girl presented with suspected mosaic trisomy 21. Birth-weight, length and head circumference were in the normal range. On day 2, she showed severe agitation, vomiting and failure to pass meconium. Hirschsprung disease (HSCR) was suspected and proven by rectum biopsy. Furthermore the girl exhibited minor craniofacial dysmorphism, muscular hypotonia and a delayed motor development.

In conventional chromosome analysis a deletion of the long arm of chromosome 13 was suspected. Array CGH analysis uncovered an interstitial 16.3 Mb deletion of 13q21.33-q31.1 encompassing 29 genes, including the endothelin-B receptor-gene *EDNRB*. The deletion was confirmed in the child and excluded in both parents by FISH.

HSCR may occur isolated or with associated features (syndromic). About 12% of HSCR are due to chromosomal abnormalities, mainly trisomy 21. In a few cases, deletions have been described (2q, 4p, 10q, 13q, 17q). Additionally, mutations in a number of genes have been found in association with HSCR.

Up to 7% of patients with isolated Hirschsprung disease have *EDNRB* mutations. Moreover, mutations in the functionally related genes *EDN3* and *ECE1* are known causes of HSCR. The pathway is involved in migration and differentiation of neural crest derivatives. Interestingly, biallelic or monoallelic mutations in *EDNRB* may also cause a subtype of Waardenburg syndrome, which is clinically characterized by additional pigmentary changes and sensorineural deafness.

Our case illustrates the particular importance of integrating array CGH analysis in the diagnostic procedures in HSCR, especially in children with additional findings like developmental delay.

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E-P11.25
Expanding the phenotype of cerebellar-facial-dental syndrome: two siblings with novel homozygous mutation in *BRF1*

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Introduction: In 2015, Borck et al. identified a novel syndrome caused by bialellic mutations in the *BRF1* gene. Clinical characteristics of patients reported to date include: intellectual disability, microcephaly, cerebellar hypoplasia, dysmorphic features (sparse eyebrows, wave-shaped palpebral fissures, low-set ears, malocclusion and prominent upper incisors) and short stature.

Material and Methods: Here we report two siblings of a consanguineous family with congenital microcephaly, growth retardation and prenatal cardiopathy. Severe global developmental delay was soon detected after birth. Brain MRI detected microcephaly with gyral simplification and

Mondini dysplasia. Both siblings also presented horse-shoe kidney and neurosensorial hypoacusis. Molecular karyotype of both siblings was normal. Exome sequencing was used as a diagnostic tool for the older sibling and Sanger sequencing for familial segregation.

Results: Exome sequencing identified a homozygous missense variant (p.Trp103Cys) in *BRF1*. Sanger sequencing detected the same variant in homozygous state also in the affected brother. Each variant was inherited from both healthy parents and a healthy sister was also carrier of one variant. No other candidate variants have been found.

Conclusions: We identified a novel missense mutation in *BRF1* in two siblings with phenotypic features of cerebellar-facial-dental syndrome. Growth, developmental delay, microcephaly and dysmorphic features are consistent with patients reported to date. We expand the phenotype of *BRF1* patients by adding Mondini dysplasia, hypoacusis and also kidney malformation.

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E-P11.28

A *de novo* mutation in *KCNQ3* gene associated with developmental delay and Dandy-Walker anomaly

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We describe the case of a 23 years old girl, born from healthy, non-consanguineous parents, presenting with developmental delay (DD), borderline intellectual disability (IQ tot 76, evaluated at 12 years of age by WISC-R), Dandy-Walker anomaly, epileptic seizures during sleep, and other minor signs. Karyotype, Fragile X and array-CGH investigations were negative.

Whole Exome Sequencing (WES) was performed on patient, her parents and her healthy brother, using the Sure Select QXT Whole Exome kit (Agilent Technologies) on NextSeq 550 instrument (Illumina).

The analysis revealed the presence of the *de novo* c.680 G>A missense variant in the *KCNQ3* gene, leading to the p. R227Q aminoacid substitution in the transmembrane domain. This variant is classified as damaging by several *in silico* prediction tools (such as Mutation Taster, Sift, Provean) and was already described as developmental disorder causing (McRae,J.F. et al., Nature 542, 433–438 (2017)).

KCNQ3 is widely expressed in the brain and encodes a subunit of a voltage-gated potassium channel, which regulates neuronal excitability. *KCNQ3*-related disorders include intellectual disability with or without seizures and/or cortical visual impairment, benign familial neonatal/infantile epilepsy. Recently, additional phenotypes, including epileptic encephalopathy and developmental delay have been described in few patients showing other missense mutations in *KCNQ3* (Fusco et al., EJPN 19.1, 102-103 (2015)).

Since little clinical information on the affected individuals is available, the clinical presentation of patients carrying *KCNQ3* pathogenetic variants remains to be fully defined.

Our report expands of the clinical spectrum related to *KCNQ3* mutations, including mild DD, Dandy-Walker anomaly and epileptic anomalies.

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E-P11.29

Kleefstra syndrome: a case report of a rare genetic disorder with an uncommon presentation

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Introduction: Kleefstra syndrome (Ks) is characterized by developmental delay, intellectual disability, hypotonia and distinctive facial features. A complex spectrum of other findings can be observed including cardiovascular and genitourinary anomalies, severe respiratory infections and seizures.

Case Report: A female neonate was born to a 40-year-old mother at 37 6/7 weeks gestation with somatometry within the normal range. On physical examination, facial dysmorphic features were noted (arched eyebrows, upslanted palpebral fissures, hypertelorism, short nose, anteverted nares, thin vermilion of the upper lip with cupid's bow, protruding tongue and macroglossia) in addition to discrete axial hypotonia and III/VI systolic ejection murmur. She had feeding difficulties and presented with multiple self-limited episodes of hyporeactivity, perioral cyanosis and desaturation during meals (minimum SpO2 level of 70%). No choking, cough, stridor, respiratory difficulty signs or hyperhidrosis were present. Septic screening and transfontanellar ultrasound were normal.

Cerebral MRI showed a thin corpus callosum with short anteroposterior length. Echocardiogram showed a dysplastic pulmonary valve and an interauricular septal defect. Indirect signs of gastroesophageal reflux were evident on nasolaringofibroscopy and respective treatment was initiated in association with speech therapy. Array-CGH identified a 754.07 Kb deletion at 9q34.3 comprising the *EHMT1* gene associated to Ks (OMIM #610253).

Conclusions: The first signs of Ks patients can be incredibly diverse making the diagnosis challenging. In this case, the early diagnosis allowed prompt evaluation by a multidisciplinary team including paediatricians and geneticists. Early referral to speech, physical and occupational therapy is also an essential part of ongoing paediatric care of these children.

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E-P11.30
KLHL7 variants causing PERCHING: one additional family and delineation of the fetal phenotype

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Recently, patients with overlapping features of Bohring Opitz Syndrome (BOPS—MIM#605039) and Crisponi Syndrome/ Cold-Induced sweating syndrome-1 (CS/CISS1—MIM#272430) were described. BOPS associates Intra-Uterine Growth Retardation (IUGR), joint contractures, microcephaly, cerebral abnormalities and dysmorphic features. The condition is usually due to *de novo* heterozygous variants of *ASXL3*. CS/CISS1 comprises also joint contractures and cerebral abnormalities. Patients with BOPS and CS/CISS1 overlapping features carry variants in *KLHL7*, bringing arguments to introduce a new spectrum for which the PERCHING acronym was suggested. *KLHL7* encodes a BTB-Kelch-related protein involved in proteasome-mediated degradation, which is highly expressed during brain development. Here we report an additional family suggestive of PERCHING and we give insight into the fetal phenotype of the condition. First patient was born from healthy unrelated parents: IUGR with polyhydroamnios was diagnosed at 25 weeks of amenorrhea. At birth, he had contracture of joints, short stature, microcephaly and dysmorphic features. MRI showed hypoplastic brain stem and a thin corpus callosum. Recurrence of the condition was identified for the second pregnancy, leading to interruption. Ultra-sound scan identified IUGR and cleft lip and palate. Pathology showed contracture of joints and extremities, cerebral abnormalities with hypopolastic brain stem and cerebellum. Whole-exome sequencing (WES) trio was launched and identified *KLHL7* composite heterozygous variants c.1124A>G; p.(His375Arg) and c.907C>T; p.(Arg303*). Both cases confirm the possible overlap of BOPS and CS/CISS1 features in patient with biallelic variants in KLHL7. The report improves delineation of PERCHING with the description of prenatal and fetal features of the condition.

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E-P11.31
Uterine and ovarian agenesis in a girl with LIG4 syndrome

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Introduction: DNA ligase IV deficiency OMIM606593 (LIG4 syndrome), is a rare autosomal recessive disorder associating lower production of T and B lymphocytes leading to severe combined immunodeficiency (SCID) B- T- NK+ resulting from misrepair of DNA doublestrand breaks by the Non Homologous End-Joining (NHEJ) pathway. Patients exhibit microcephaly, "bird like" appearance, growth retardation, cognitive disorders, combined immune deficiency. The clinical spectrum is broad, ranging from childhood lethality to mild presentations without hematological disorders. To date, 30 patients have been reported in the literature. Here, we report the observation of a 18 years old girl with LIG4 syndrome with mild immunohematologic features but severe congenital malformations of the genital tract.

Methods: The patient was seen at 7 years of age in genetic center for growth failure (−3 SD), severe microcephaly (−5 SD), facial dysmorphism and mild learning

disability. Growth hormone therapy was given without efficacy. Pubertal delay was investigated at 14 years of age and led to the diagnosis of uterine and ovarian agenesis. Routine haematologic survey revealed thrombocytopenia, lymphopenia and macrocytosis.

Results: Lymphocyte subsets showed combined immunodeficiency with profound T- and B- lymphocytopenia without clinical expression. Radiosensitivity was showed on cultured fibroblasts and exome sequencing revealed a homozygous known pathogenic variation in *LIG4*: c.2440C>T p. (Arg814*). Parents were heterozygous for the variation.

Conclusion: Female genital tract anomalies have never been reported in patients with LIG4 syndrome. Further studies are needed to explore mechanism(s) explaining both embryonic Mullerian duct anomalies and bilateral ovarian agenesis which are very rare in association.

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E-P11.32
Investigation of FBN1 gene mutations in clinically diagnosed Marfan's syndrome patients

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Objective: Marfan's syndrome (MFS) is an autosomal dominant type 1 fibrillinopathy that can affect eye, skeletal and cardiovascular systems. MFS is associated with mutations in the Fibrillin 1 gene (*FBN1*) which is located at 15q21.1. Generally revised Ghent criteria is used in the diagnosis at MFS due to high clinical heterogeneity. Estimated prevalence of MFS is about 1/3000–1/10000. In this study, we aimed to investigate the *FBN1* gene variants and their phenotype-genotype correlations in clinically diagnosed MFS patients.

Materials and Methods: Ten clinically MFS diagnosed cases were referred to our molecular laboratory for molecular analysis between January 2018 and August 2018. Whole *FBN1* gene exons and exon-intron boundaries were sequenced using targeted new generation sequencing platform (Miseq, Illumina). Detected variants which were interpreted to be disease-causing were evaluated in terms of clinical features and phenotype-genotype correlation.

Results: Disease-causing *FBN1* gene variants were detected in 8 of 10 cases. Among 8 variants 4 were previously described (c.2585G>A, c.529T>C, c.6037 +2T>C, c.1633C>T) and remaining 4 variants were novel (c.6821G>T, c.1528_1531dup, c.7027delG, c.5077_5078insT). Genotype phenotype correlation studies for mutations detected were found to be compatible with the previous studies.

Conclusion: This is the largest study on *FBN1* mutation spectrum and phenotype-genotype correlation in MFS patients reported from the Turkish population. Furthermore, this study contribute to the literature with four novel mutations. Detection of disease-causing variants in patients with MFS will contribute to the development of diagnostic and follow-up procedures, prevention of complications and offering prenatal and preimplantation genetic diagnosis options to families.

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E-P11.33
Neonatal death expands the phenotype of megalencephaly capillary malformation syndrome with transient hypoinsulinaemic, hypoketotic hypoglycaemia due to a *PIK3CA* mutation

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Introduction: *PIK3CA* mosaic activating mutations cause Megalencephaly Capillary Malformation syndrome (MCAP)/segmental overgrowth. *AKT2* activating mutations cause hypoinsulinaemic, hypoketotic hypoglycaemia (HIHKH). PIK3CA and AKT2 are part of the insulin-signaling pathway, PIK3CA acts upstream of AKT2. Recently, HIHKH was reported in four patients with *PIK3CA* mutations. We describe the fifth patient.

Patient and Methods: A Caucasian girl with healthy non-consanguineous parents was born by gestational age 38 +4 weeks, birth weight 4.940g, length 54cm, head circumference 37cm, all +2½-3SD. Dysmorphic features included lateralized right-sided overgrowth especially of the face, a distinct capillary malformation on the upper lip/philtrum and into the nasal and oral cavities, generalized

cutaneous capillary malformations on the body and nail hypoplasia. Hypoglycaemia (blood glucose 0.9mmol/L) occurred within two hours after birth. Low plasma calcium and treatable seizures developed. She had generalized hypotonia and hypermobility. Severe respiratory insufficiency required artificial ventilation. Active treatment was discontinued, and she died 18 days old.

Results: MRI of the cerebrum showed right-sided hemimegencephaly, polymicrogyria, corpus callosum hypoplasia. Abdominal ultrasound showed enlargement of the right kidney. During hypoglycaemia (1.4 mmol/L) she had suppressed ketones (0.2 mmol/L), decreased C-peptide (133 pmol/L) and normal proinsulin (6.5 pmol/L), consistent with HIHKH, which resolved by age 12 days. Pituitary hormone analyses were normal. Lymphocyte DNA analysis showed a mosaic known heterozygous pathogenic *PIK3CA* mutation c.1357G>A; p.Glu453Lys in 18% sequencing reads. Molecular results of fibroblasts and Achilles biopsies are pending.

Conclusions: Our patient with transient HIHKH and MCAP syndrome due to a mosaic *PIK3CA* mutation expands the phenotype to neonatal lethality.

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E-P11.34
Miller-Dieker syndrome: different phenotypes in 5 cases

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Miller-Dieker Syndrome (MDS) is a continuous gene microdeletion syndrome of chromosomal region 17p13.3 associated with abnormal neuronal migration. The typical phenotype includes severe developmental abnormalities, lissencephaly, growth delay, and cranial-facial dysmorphism. We present five unrelated, sporadic cases with MDS (two boys, three girls—aged between 1 and 11 years old), with the aim of illustrating the particularities identified. All cases were confirmed using MLPA Kit P064, Kit P249. Case 1: marked postnatal growth retardation, severe neurodevelopment delay, microcephaly, typical dysmorphic facies, muscular spasticity and hypotonia, lissencephaly, agenesis of corpus callosum, seizures, ichthyosis, varus equine; Case 2: severe neurodevelopment delay, staturo-ponderal hypotrophy, microcephaly, typical facies, palatoschisis, congenital cardiac anomalies; Case 3: moderate neurodevelopment delay, microcephaly, non-characteristic facial dysmorphia, low weight, hypotonia, pachygyria, seizures, cryptorchidism, laxity of connective tissue; Case 4: severe neurodevelopment delay, staturo-ponderal hypotrophy, microcephaly, typical dysmorphic facies, hypotonia, lissencephaly, optical nerve atrophy, nystagmus, hepatic vascular malformation, scoliosis. Case 5, a more complex genetic anomaly, (17p13.3 deletion associated with a 2q duplication), features severe neurodevelopment delay, low weight, characteristic dysmorphic facies, bilateral cleft lip, spina bifida occulta. 2/5 cases presented no structural malformation of the brain. This report highlights the particular features of each presented case and emphasizes the high variability of MDS phenotype in patients with similar ages.

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E-P11.35
The mosaicism radio of 45,X/46XY is the reason of mixed gonadald dysgenesis for phenotype

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Background: Patients with mixed gonadal dysgenesis (MGD), whose prototypical karyotype is 45,X/46,XY, are known to manifest complications characteristic of Turner syndrome.

Objectives: We report a 11-year-old social female with MGD presenting Turner syndrome. *Clinical signs*: Clitoris enlargement, normal uterus. Ultrasounds: Do not observe testides on either side. There is no herniation on either side. No ovaries clearly visible.

Methods: Sterile peripheral blood with heparin for anticoagulation is cultured in the RPMI medium in 72 h, harvested and performed the G—banding. The metaphase chromosome is captured by the Karl Zeiss microscope system, analysed by Ikaros software (Metasystem) following the ISCN guidelines. FISH test:Fluorecence in situ hybrydization.Interphase FISH.Probe: SEX(DXZ1)/SEY (DYZ3) Dual Probe.TDF(testis determining factor):Using multiplex PCR technique to detect the absence or mutations in SRY gene.

Results: Chromosomal analysis:the karyotype 45,X[3]/46,X,dic(Y;22)(p11.3;q13.3)[27] (named 45,X/46,X+Y fragment).The ratio of mosaicism in the interphases was estimated by FISH with probes to identify the X centromere-specific repeat sequence and Yp11.2. The mosaicism ratio of 45,X/46,X+Y fragment: 8,53%/91,47%.TDF (testis determiner testing) is positive.

Conclusions: We recommend girl with otherwise unexplained short stature, being short for their families, should be karyotyped routinely as is recommended in short-stature girls. Boys with 45,X/46,XY mosaicism require a thorough clinical evaluation similar to that performed in girls with Turner syndrome and must be routinely followed up for their potential to respond favorably to GH treatment and for late onset abnormalities, such as infertility and gonadal tumors.

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E-P11.36
Identification of a new mutation in ZEB2 gene in a Ukrainian girl with Mowat- Wilson Syndrome

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Mowat-Wilson syndrome, MWS is a rare (1 to 50.000–100.000) autosomal dominant disease caused by *ZEB2* gene mutations which as usual occurs *de novo*. Phenotypic variability makes difficult to diagnose MWS.

Materials and Methods: The case of MWS syndrome which was suspected prenatally is described. The NGS analysis of *ZEB2* gene and next conformation Sanger sequencing was performed after birth.

Result: The polyhydramnios, heart defect (ventricular septal defect, dextroposition of the aorta), brain and renal abnormalities (agenesis of the corpus callosum; duplex kidney, hydronephrosis) were diagnosed prenatally in the first pregnancy of healthy parents. It was full-term birth with 3.25kg weight, 54cm length, 7/8 Apgar score. The girl was referred to genetic counselling. Among congenital anomalies were heart defects:tetralogy of fallot, pulmonic valve and pulmonary artery stenosis, patent dustus arteriosus, tricuspid insufficiency; brain- agenesis of the corpus callosum; renal- cyst to20 mm in the left kidney. The child had pointed chin, cupper ears, fleshy upturned lobules, hypertelorism, deep-set eyes, downslantinng palpebral fissures, broad eyes, wide nasal bridge, prominent nasal tip, hypotonia. The genetic testing revealed a previously unpublished sequence variant c.493G>T (p.Glu165*) in *ZEB2*. This sequence change creates a premature translational stop signal (p.Glu165*). This variant is not present in population databases and not been reported in the literature. The sequencing of *ZEB2* gene of both parents is processing.

Conclusions: The data about MWS is insufficient. Functional studies and more reports are required to understand the effect of the mutation and genotype-phenotype correlation.

Y. Sharhorodska: None. **M. Dushar:** None. **N. Prokopchuk:** None. **H. Akopyan:** None. **H. Makukh:** None.

E-P11.38
Rapid and optimal diagnosis in malformative syndromes at newborns

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Introduction: Congenital malformations (minor or major) represent one of the prioritary problems in neonatal pathology and require a multi-disciplinary approach: neonatologist, genetician, cardiologist, neurologist and often require surgical intervention.

Objectives: The authors propose an analysis of the complexity of malformations, correlations between prenatal and postnatal diagnosis, immediate and distant morbidity and mortality.

Material and method: The study group included 43 newborns with malformations hospitalized in our Clinic; it was conducted over a period of 12 months.

Results and discussions: Most of the patients came from uncontrolled pregnancies—70%, 25% from partially controlled and 5% from controlled pregnancies and plurimalformative syndrome was diagnosed prenatally. 7(16,27%) presented plurimalformative syndrome, 5 (11,62%) deceased due to malformations complexity. The most frequent malformations: heart and big blood vessels congenital malformations—20(46,51%), from which 6 (13,95%) were diagnosed with severe congenital heart disease by cardiac ultrasonography and angio MRI. Mielomeningocel associated with Arnold Chiari syndrome and hydrocephaly was present in 10 (23,25%) cases, at 1 (2,32%) case was observed lumbar mielomeningocel without cerebral malformations. In 2(4,65%) cases were present cerebral malformations. Postnatal diagnosis was determined by ultrasound and/or by cerebral MRI. In 3(6,97%) cases were present renal malformations In all the cases genetic consult and investigations were done.

Conclusions: In the malformative syndrome interdisciplinary team cooperation is very important. It is also very

important the pregnancy monitoring and congenital malformations intrauterine diagnosis. Genetic consult and antenatal diagnosis can lead to early management in therapeutic process, favorable prognostic and minimal further complications.

A. Manea: None. **I. Jurca-Simina:** None. **D. Cioboata:** None. **O. Costescu:** None. **F. Doandes:** None. **N. Lungu:** None. **M. Boia:** None.

E-P11.39

TWO TURKISH CASES with NICOLAIDES-BARAITSER SYNDROME PRESENTING A NOVEL *SMARCA2* VARIANT

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Nicolaides-Baraitser Syndrome (NCBRS) (OMIM#601358) is an uncommon and well recognized autosomal dominant entity that is characterized by sparse scalp hair, characteristic coarse facies, microcephaly, seizures, developmental delay, intellectual disability and prominence of the interphalangeal joints and distal phalanges. Seizures are also common finding besides developmental delay and intellectual disability (ID) which is severe approximately in half, moderate in one third, and mild in the remainder. About one third of the affected patients never develop speech or language skills. Clinical diagnosis of NCBRS is often challenging because of its progressive course. It is known that there is a continuum of phenotypic spectrum as well a distinction between the classic or severe phenotype and the atypical or mild form. The prevalence is not known; however, to date, around one hundred patients have been described in the literature worldwide. The delineation of the phenotype of Nicolaides-Baraitser syndrome (NBS) and the identification of causative heterozygous missense mutations in the *SMARCA2* has been made recently. Here, we report two Turkish patients with NCBRS. To the best of our knowledge, this is the first case report of this syndrome from Turkey with a confirmed molecular diagnosis and the novel variant [NM_003070.5:c.3389G>T p.(Gly1130Val)] is correlated with a mild-moderate phenotype. The other one is [NM_003070.5:c.2554G>A p.(Glu852Lys)] a known variant correlated with severe phenotype.

K. Karaer: None.

E-P11.40

The case report of a patient with Niemann Pick disease: reality in Brazil

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Background: Niemann Pick disease consists of a genetic condition, recessive and degenerative, characterized by mutations in the NPC-1 gene, which is located on chromosome 18 (90 to 95% of cases) and the gene, NPC-2, on the chromosome 14. The symptomatology is: hepatosplenomegaly, neonatal jaundice, pulmonary infiltrates, supranuclear palsy of the vertical eye, ataxia, cognitive dysfunction, dysphagia, dysarthria, dystonia, gaseous cataplexy, and seizures. To diagnose it is necessary to perform tests with the biomarker, which consists of the dosage of oxysteroids and lysosphingolipids. In addition, a skin biopsy is performed (Filipin's test). However, confirmation is the genetic test. Until then, the only treatment is Miglustate (Zavesca)—TRS.

Objective: To report a clinical case of a patient presenting Niemann Pick Type C disease, with neurological manifestations.

Case Study: Patient MRFS, 13 years, female, gestational history and delivery without complications, and until the beginning of 2012 did not present any neuropsychomotor impairment. Magnetic resonance imaging of the brain, enzyme dosing for Tay Sachs diseases, Neuronal Lipofuscinosis, first-line screening for EIM (inborn errors of metabolism, which were normal) and NPC tests was performed. Filipin, tested positive, concluding the patient's diagnosis.

Final Considerations: The case reported demonstrates the wide symptomatology of the disease, which can be mitigated or delayed due to an early diagnosis, very difficult due to the lack of support in the country.

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E-P11.41

Evolution in time and hearing impairment in oculo-auriculo-vertebral spectrum

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Oculo-auriculo-vertebral spectrum (OAVS) is a rare disorder characterized by defects of aural, oral, mandibular and vertebral development, with a reported prevalence of up to 1/3.500 births. Ear anomalies including microtia, external auditory canal atresia and eustachian tube dysfunction cause both conductive and sensorineural hearing loss. Both genetic and environmental factors are thought to contribute to this craniofacial condition, however, the mechanisms are still poorly understood. We have performed a clinical study on 10 cases of OAVS diagnosed in Iasi Medical Genetics Center, aiming to identify defects associated to the main features, clinical evolution and to correlate clinical features with hearing loss. Our group included 5 males and 5 females. All cases have been sporadic and result from uneventful pregnancies. All ten cases had facial asymmetry (6/10 right hypoplasia). Major clinical findings include: microtia/anotia 10/10, preauricular tags 10/10, external auditory canal atresia 8/10, conductive hearing loss 8/10, mixed hearing loss 1/10, dental malposition 8/10, vertebral malsegmentation 4/10. Occasional findings include: congenital heart defect, epilepsy and talasemia minor. Genetic tests have been normal. The evolution in time has been constant. In conclusion, we present the evolution in time and the importance of a multidisciplinary approach in the management of the patients. Clinical features at different ages will be illustrated. Hearing impairment is variable in patients with OAVS and often there is correlation with ear abnormalities. The evaluation of these structures is important in the management of individuals with OAVS. Early diagnosis and intervention have made significant improvements, especially for hearing loss.

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E-P11.42
Oral-facial-digital syndrome type 1: further clinical and molecular delineation in three new families

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Introduction: Oral-facial-digital syndrome type 1 (OFD1) is a rare genetic disorder associated with congenital anomalies of the oral cavity, face and digits. Affected OFD1 individuals may also exhibit multiple congenital malformations affecting their renal, dermatological and central nervous systems. OFD1 belongs to the group of genetic diseases known as ciliopathies. It is associated with mutations in the *OFD1* gene.

Materials and Methods: We investigated four affected patients from three unrelated families with a clinical diagnosis of OFD1 who lacked molecular genetic confirmation, using a variety of sequencing techniques.

Results: We discovered a novel pathogenic heterozygous missense mutation c.635G>C (p.Arg212Pro) in the *OFD1* gene in two affected patients of one family. A heterozygous splice-site mutation, intron 10 c.1056-2A>G in *OFD1* was detected in the affected patient of the second family. A novel frameshift, loss of function mutation c.306delA (p. Glu103LysfsTer42) was detected in the affected patient in the third family, who has polycystic kidney disease which is clinically characteristic of OFD1.

Conclusions: The clinical findings of our study confirm the variable phenotypic presentation of OFD1, broadening our understanding of the features associated with this syndrome. The genetic variants discovered will add to the spectrum of known *OFD1* mutations. Molecular diagnostic confirmation demonstrated in our patients has positive implications and will lead to improvements in clinical management, which include imaging surveillance of systemic manifestations and genetic counselling.

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E-P11.43
Molecular cytogenetic and clinical assessment in a new case of partial trisomy 9

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Partial trisomy 9 is a rare condition associated with variable phenotype including: typical cranio-facial features, intellectual disability, developmental delay, skeletal and cardiac abnormalities. The severity of phenotype is related to the extent of the triplicated chromosome region. We report on 1,6-year-old girl with dysmorphic features, global developmental delay, brain anomalies and skeletal defects. The pregnancy was uneventful. At 21 weeks of pregnancy fetal ultrasound revealed dilated ventricles and fetal MRI, performed at 24 weeks confirmed venticulomegaly. The patient was born at 39 weeks by C-section. Physical parameters at birth were normal. She required 2 days in intensive care unit. Clinical exam at 1.6-years-old revealed

brachycephaly, suggestive cranio-facial appearance, strabismus, bilateral simian crest, camptodactyly of the 2nd finger, clinodactily of the 5th finger, pectus excavatum, left hip dislocation, developmental delay. Cytogenetic analyses for the proband and her parents were performed. The patient's karyotype showed an extra-chromosome representing +der(9) and the result of arrayCGH was arr [GRCh37] 9p24.3q22.1(208454_91446674)x3. The father has a normal chromosomal structure while the mother carried a balanced translocation between chromosome 9 and 14. The presence of maternal translocation suggested a 3:1 segregation pattern as the underlying mechanism for the patient's chromosomal abnormalities. Only 2 cases with pure partial trisomy 9 and similar size triplicated material, but no molecular studies have been described in literature so far, both dying in the first month of life. Comparing the phenotype of our patient along with the data obtained using molecular cytogenetic approach with previous reports could contribute in a better genotype-phenotype delineation.

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E-P11.44

A novel loss of function deletion in *PIEZO1* cause autosomal recessive lethal generalized lymphatic dysplasia

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Introduction: Piezo-type mechanosensitive ion channel component 1 (*PIEZO1*) encode a pore-forming subunit of a cation channel that function as a mechanotransducer involved in the vascular system development. Previously, homozygous or compound heterozygous loss of function mutations in *PIEZO1* have been described in 17 patients, causing autosomal recessive congenital generalized lymphatic dysplasia (GLD) affecting the lymphatic system development.

Materials and methods: Whole exome sequencing, Sanger sequencing and bioinformatics analysis were used to genetically characterize a pair of monochorionic diamniotic (MCDA) twins, presenting with variable features of autosomal recessive congenital GLD consistent with symptoms previously described. Blood of the heterozygous parents are currently being examined using RNA sequencing, blood smear and erythrocyte size measurements, investigating the affected alleles association to occasional stomatocytes, spherocytes and Piezo1 function. To investigate the lymphatic system and etiological diagnosis of oedema, immunohistochemistry will be performed on fetus material using antibodies towards CD31, CD34, smooth muscle actin antibody, D2-40.

Results: A novel homozygous loss of function *PIEZO1* deletion (c.401_404del;p.134_135del, NM_001142864) was identified through whole exome sequencing and variant analysis. The variant has not been reported in gnomAD or 1000genomes. MutationTaster predicted the variant to have a deleterious effect on the protein function, with frameshift suggestively resulting in a stopcodon (V134Afs71).

Conclusion: We report on a pair of monochorionic diamniotic (MCDA) twins with a novel *PIEZO1* mutation. Functional studies will be performed to evaluate the pathogenicity of the mutation.

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E-P11.45

A patient with PIK3CA-related overgrowth syndrome (PROS) with prenatal and postnatal findings

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The mosaic activating mutation in gene PIK3CA causes the development of an overgrowth syndrome (PROS) which is described as CLOVES (Congenital Lipomatous asymmetric Overgrowth of the trunk with lymphatic, capillary, venous, and combined-type Vascular malformations, Epidermal naevi, Scoliosis/Skeletal and spinal anomalies) This mutation gives rise to abnormal PI3K-AKT-mTOR pathway activation. The eleven months old girl was referred to us due to macrocephaly, dysmorphic characteristics and abnormal prenatal findings. She had macrosomia, macrocephaly, ventriculomegaly and polydactly on prenatal ultrasonography. A diagnostic amniocentesis showed a normal female karyotype. The physical exam showed her weight was 15kg (>97th centile), length 87 cm (50–75th centile) and head circumference 59 cm (> 97th centile). She had macrocephaly, frontal bossing, bilateral polydactly of both hands and developmental delay. Her parents were not related and family history was unremarkable. On laboratory

examination, FISH analysis for Sotos syndrome was normal. Whole exome sequencing showed pathogenic variant for PIK3CA (c.2740 G>A p.(Gly914Arg)) This variant has previously been linked to Megalencephaly-capillary malformation disease in the literature. The aim of this presentation is to emphasize that in patients who have macrosomia and the other characteristic features as defined above on prenatal ultrasonography, PROS should be kept in mind in the differential diagnosis.

E. Mihci: None. **M. Şimsek:** None. **B. Nur:** None.

E-P11.46
FLOPPY AND DISMORPHIC NEONATE ASSOCIATED WITH TWO DISTINCT PATHOGENIC CNV

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Copy number variants (CNV) are a major cause of neurodevelopment disorders. Several recurrent variants have already been described. Co-existence of different recurrent variants in the same patient, although rare, can be expected due to the frequency of CNV and the existence of balanced rearrangements as the origin of the CNV. We present a case of a 3 mo girl with the clinical presentation of late preterm birth, hypotonia, craniofacial dysmorphia. Family history revealed a maternal uncle with Angelman syndrome, and another maternal uncle that died in neonatal period from congenital cardiopathy. Array CGH was performed using Cytoscan 750K (Applied Biosystems). Results revealed a duplication of 4,050 Mbp at 15q11.2q12 and a deletion of 3,424 Mbp at 22q11.1q11.21. Both variants were classified as pathogenic and are associate with: chromosome 15q11-q13 duplication syndrome (OMIM 608636) and the chromosome 22q11.2 deletion syndrome (OMIM 188400).Cytogenetic tests performed both on the index case and the mother, confirmed that the mother is a carrier of a balanced translocation between chromosomes 15 and 22 (breakpoints 15q11.2 and 22q11.2), and that the index has inherited an unbalanced form of the translocation. Testing of the maternal uncle also confirmed that is was also a carrier of an unbalanced form of the translocation. This case confirms that structural chromosomal rearrangements are an important mechanism of CNV, and that both array CGH and cytogenetic testing are critical tools to detect the families at risk and provide the appropriated genetic counselling and follow-up.

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E-P11.47
Clinical and molecular aspects of *PTEN* mutations in pediatric population: A retrospective study

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Introduction: *PTEN* is a tumor suppressor gene localized at 10q23.31. *PTEN* gene defects are responsible for the *PTEN* hamartoma tumor syndromes (PHTS) including Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, *PTEN*-related Proteus syndrome and Proteus-like syndrome. In this study, we present clinical and molecular findings of patients with mutation in the *PTEN* gene. Our aim is to define most common findings to contribute phenotype-genotype correlation.

Methods: Ten PHTS patients from 7 families who were referred to our outpatient clinic between 2010 and 2018 and confirmed with clinical findings and molecular tests were included in the study. Clinical findings, laboratory and imaging tests results were obtained from hospital records. Sequencing of *PTEN* gene was performed. Variant interpretation was done in accordance with ACMG recommendations.

Results: Of 10 patients described, 6 were males and 4 were females. Macrocephaly is the most common clinical finding this was followed by neurodevelopmental delay (6 patients), skin lesions (5 patients) and pathologic cranial MRI findings (4 patients). Seven different heterozygous *PTEN* gene variants were found in 7 families. Four of these were located in Exon 5 which was described as a hotspot area for *PTEN* gene. Mutation types were missense (3), frameshift (2), splice site (1) and nonsense (1) mutations.

Four mutations (c.381_385delAAAGG, c.345_346insTG, c.417A>T, and c.746T>G) were novel.

Conclusion: Although genotype-phenotype correlation of PHTS is not well-described, screening of *PTEN* gene mutations in patients with macrocephaly is recommended due to increased risk of cancer. Four mutations were reported for the first time in this study.

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E-P11.48

Iaşi Regional Genetics Centre's experience with diagnosing RASopathies

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Introduction: Noonan syndrome (NS) belongs to a group of conditions collectively known as RASopathies. Patients affected by NS have particular facial features (deep philtrum, widely spaced eyes, low-set and posteriorly rotated ears), short stature, webbed neck, skeletal abnormalities (*pectus excavatum/carinatum*) and heart defects (most often pulmonary valve stenosis).

Most commonly, mutations in NS arise in the *PTPN11* gene (approximately 50%). Other mutations have been cited in *SOS1* (around 15%), *RAF1* and *RIT1* genes, the latter two accounting for less than 5% of cases. Many other genes have been rarely associated with NS.

Materials and Methods: Since we only started genetic testing for RASopathies and NGS is still widely unavailable, we selected 14 patients, based on the NS diagnostic score, and performed Sanger sequencing of all the exons of the *PTPN11* gene. Patients who tested negative for mutations in the PTPN11 gene will further undergo an NGS panel testing for RASopathies.

Results: Exon mutations in *PTPN11* gene were found in only 3 cases of the total 14. These mutations have been commonly cited as pathogenic for NS: *c.215C>G, c.417G>C, c. 1483C>T* (*NM_002834.3)*. We also report one apparently benign intronic variant (*rs.184804143g. 36206 C>A)*.

Conclusions: We attempt to discuss the clinical features of patients with PTPN11 mutations and of those without it (genotype-phenotype correlation). We also discuss the possibility of a lower prevalence of mutations in the *PTPN11* gene in the Eastern European area, in favour of mutations in the other genes described above.

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E-P11.50

Mosaic Inverted Duplication Deletion Ring Chromosome 4: case report

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We report 34-year-old male presenting with several congenital malformations at birth. The baby was delivered at 36 weeks' gestation and weighted 1900g. Our patient presented with development delay, growth retardation and intellectual delay. At 6-year-old he had many facial dysmorphisms including microcephaly, microretrognathia, mongoloid palpebral fissures, strabismus, prominent and aquiline nose, long flat philtrum and ogival palate. Bilateral cryptorchidism, kyphoscoliosis, esophageal stenosis, clinodactyly, atopic dermatitis and congenital hypothyroidism were also present. Nowadays his small size is striking.

The CytoScan array 750K, GTG-banded karyotype, subtelomere and targeted FISH were performed to the patient.

The array revealed 4p16.3 deletion and 4p16.3p14 duplication. Deleted region was 1556kbp including 16 OMIM genes, not including WHS gene, whereas duplicated region was 37767kbp including 101 OMIM genes. The karyotype of the patient showed a ring chromosome 4 in mosaicism, 46,XY,r(4)[86]/46,XY[14]. Metaphase FISH of telomere regions was performed to confirm the 4p telomere deletion at the ring(4). We designed targeted FISH probes to see in metaphase how was disposed the duplicated region and we observed that it was inverted. At the normal cell line both telomeres were intact and the targeted hybridization pattern was normal.

U-type exchange is the most frequent mechanism proposed to explain InvDupDel rearrangement and the ring formation is described as a possible consequent rescue mechanism. As far as we know this is the first case of mosaic 46,XY,InvDupDelRing(4)/46,XY described in the literature. We need further investigation to elucidate the mechanism of the presence of the apparently normal cell line.

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E-P11.51

Novel mutations in *RIPK4* underlie ectodermal dysplasia-syndactyly-keratoderma syndrome

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Introduction: Ectodermal dysplasias form a large group of hereditary conditions with more than 200 members. We previously identified loss-of-function mutations in *PVRL4*, encoding the cell-cell adhesion molecule nectin-4, in ectodermal dysplasia-syndactyly syndrome (EDSS).

Materials and Methods: Two sibs with ectodermal dysplasia (hair and nails anomalies), cutaneous syndactyly at hands and feet and palmoplantar hyperkeratosis were analyzed by whole-exome sequencing. Data were prioritized according to recessive mode of inheritance (i.e. presence of the both allelic variants in affected individuals at the heterozygous state in the parents).

Results: No mutations were identified in *PVRL4* but two novel variants emerged in the *RIPK4* gene segregating with the disease under autosomal recessive inheritance. One of the missense variants affects the kinase domain, while the second lies in the intermediate domain preceding the ankyrin-repeats.

Conclusions: Our results broaden the clinical and molecular spectrum of *RIPK4*-related disorders to include EDSS. RIPK4 (a Serine/Threonine Kinase) was previously associated to Bartsocas-Papas (BPS) and Curly Hair-Ankyloblepharon-Nail Dysplasia (CHAND) syndromes. Both display ectodermal derivative defects such as hair anomalies, alopecia and nail dysplasia, while BPS manifests massive pterygia, oligosindactyly and is often lethal. Although *RIPK4* and *PVRL4* are involved in different cellular pathways, their alteration results in overlapping clinical manifestations.

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E-P11.52

Clinical description and mutational profile of a moroccan series of patients with Rubinstein Taybi syndrome

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Background: Rubinstein-Taybi syndrome (RSTS; OMIM 180849) is a rare autosomal dominant developmental disorder with an estimated prevalence of one case per 125,000 live births. RSTS is characterized by typical face, broad thumbs and hallux, short stature, and intellectual disability. Facial dysmorphism is characteristic with microcephaly, low frontal hairline, arched eyebrows, long eyelashes, convex profile of nose, narrow palate, and micrognathia. RSTS is mainly due to mutations or microdeletions of the *CREBBP* gene (about 60%) and more rarely of the *EP300* gene (8%).

Case presentation: We report here, the clinical and molecular data of a series of six Moroccan patients with a phenotype of RSTS. The molecular study of the major gene *CREBBP* (by Sanger Sequencing followed by CGH array if normal sequence) revealed point mutations in five patients. For the sixth patient, CGH array revealed a microdeletion carrying the *CREBBP* gene.

Conclusions: Through this work, we emphasize the importance of clinical expertise in the diagnosis, management and genetic counseling in Rubinstein Taybi syndrome.

W. Smaili: None. **S. Chafai Elaloui:** None. **J. Van Gils:** None. **P. Fergelot:** None. **I. Ratbi:** None. **M. Tajir:** None. **B. Arveiler:** None. **D. Lacombe:** None. **A. Sefiani:** None.

E-P11.53

Nephronophthisis clue for diagnosis of Senior Loken Syndrome in a patient with amaurosis

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Introduction: Senior-Loken syndrome is a rare disease with a world prevalence of 1 in 1 million people, characterized by two specific manifestations: a congenital Leber amaurosis, usually present in the first year of life and nephronophthisis—a cystic kidney disease that leads to

tubular atrophy and ultimately end stage renal disease. Our aim is to present a 17-year-old girl diagnosed at one year of age with congenital Leber amaurosis. Next admission was after 15 years for renal impairment, suggesting the presence of Senior-Loken syndrome.

Materials and Methods: The patient was clinically evaluated, performed lab tests, abdominal ultrasound and finally renal biopsy.

Results: At admittance, our patient presented sight loss, anuria, peripheral edema and fatigue. Lab tests revealed anemia, elevated BUN levels, creatinine, and modified GFR, confirming end stage renal disease. Renal ultrasound showed renal dysplasia. Renal biopsy confirmed medular cystic disease(nephronophthisis). At this moment, Senior-Loken Syndrome was suspected and genetic testing was done, which confirmed type 5 of Senior-Loken Syndrome. Renal transplantation was performed from a related donor. Now the patient is in a follow up program by a multidisciplinary team.

Conclusions: When Leber amaurosis is present, the patient has to be monitored by a nephrologist. If kidney disease is present, Senior-Loken Syndrome has to be suspected, that must be genetically confirmed. Early detection of the renal disease can delay the progression to end stage kidney disease. A multidisciplinary approach is mandatory in order to delay end stage renal disease and to offer a better quality of life.

M. Gafencu: None. **Z.G. Andrei:** None. **F.A. Chisavu:** None. **M. Puiu:** None. **A. Chirita-Emandi:** None. **N. Andreescu:** None. **R.F. Stroescu:** None. **A.M. Ardelean:** None. **G.S. Doros:** None.

E-P11.54
Is primary lymphedema in Phelan-McDermid syndrome caused by *SHANK3* haploinsufficiency?

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We report on a 5 year-old boy who developed primary lymphedema in the left lower extremity at the age of 3 years. In addition, he presents significant cognitive delay, especially concerning language, behaviour problems with stereotypies and chewing non-food items and decreased perception of pain. Currently, 28 genes have been shown to cause different forms of primary lymphedema, explaining only about 30% of the cases. Different genetic tests have been performed for our patient: molecular karyotyping, fragile-X analysis, as well as the analysis of a large panel of genes involved in lymphedema. However, all were negative.

Whole-exome sequencing was subsequently used to search for the causative gene. It allowed to identify a nucleotide deletion in the *SHANK3* gene (c.4191delC), leading to frameshift and premature truncation (p.Ser1398-Profs*30). The deletion was confirmed by Sanger sequencing and was proven to have appeared *de novo*, as none of the parents has it.

Heterozygous deletions of 22q13.3, encompassing *SHANK3*, have been associated with Phelan-McDermid syndrome. Around 3% of the patients have *SHANK3* point mutations. The main phenotypic features of the Phelan-McDermid syndrome are neonatal hypotonia, delayed or absent speech, developmental delay, intellectual disability and behaviour problems. Around 10–25% of patients with microdeletions develop lymphedema. The causal gene for lymphedema in that locus is still unknown. As our patient has *SHANK3* point mutation, we speculate that it could be the causal gene. If this is true, other genetic or non-genetic factors could be necessary for lymphedema to develop, explaining the reduced penetrance in the Phelan-McDermid syndrome.

L.C. Evenepoel: None. **P. Brouillard:** None. **N. Revencu:** None. **L. Boon:** None. **M. Vikkula:** None.

E-P11.55
A novel case of Simpson-Golabi-Behmelsyndrome caused by *GPC3* hemizygous nonsense mutation

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Introduction: Simpson-Golabi-Behmel syndrome (SGBS) is a condition that affects many parts of the body and occurs primarily in males. SGBS is an overgrowth disorder, meaning that people with the disease are larger

than average at birth (macrosomia) and continue to grow and gain weight at an unusual rate. Genomic rearrangements and point mutations involving the glypican-3 gene (*GPC3*) at Xq26 have been shown to be associated with SGBS. By using next-generation sequencing, we aimed to identify the disease-causing mutation in a boy with SGBS phenotype.

Materials and Methods: An 8-years-old boy was referred with initial symptoms including macrosomia, macroglosia, abnormalities of the palate, an extra nipple, various birth defects such as umbilical hernia, skeletal anomalies, cryptorchidism, and Wilms tumor. Intellectuality was normal. Patient's DNA was sequenced using Illumina® platform, TruSight One sequencing panel.

Results: Genetic analysis found a hemizygous transition c.712G>T in exon 3 of the glypican 3 gene, *GPC3*, corresponding to a novel mutation p.Glu238Ter. Using common bioinformatics approach, we predicted that premature stop codon resulting in a nonsense mediated decay or a truncated protein product is the possible mechanism of the disease.

Conclusions: Simpson-Golabi-Behmel type I syndrome is an overgrowth/multiple congenital anomalies syndrome caused by mutations in a semi-dominant X-linked gene *GPC3*. About 250 patients have been reported so far in the medical literature. The prevalence of the syndrome is unknown. Using next-generation sequencing technology we determined the genetic cause in another case presenting typical features for SGBS type I phenotype.

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E-P11.56
Everolimus trial in a refractory epilepsy case secondary to a PI3K-Akt-mTOR signaling pathway mosaic mutation: from physiopathological theory to clinical practice

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Introduction: Smith-Kingsmore syndrome, caused by *de novo* Mtor gene mutations, is a rare autosomal dominant syndromic intellectual disability syndrome characterized by macrocephaly, seizures and facial dysmorphic features. When the variant is present at a mosaic state, hypopigmentation along Blaschko's lines, body asymmetry and hemimegalencephaly can be observed.

Materials and methods: We report the case of a patient with Smith-Kingsmore syndrome who received everolimus treatment, a specific mTOR inhibitor. The patient, a 12-year-old-female had cognitive impairment with psychomotor regression, intractable seizures, hypopigmentation along Blaschko's lines, body asymmetry overgrowth, hemimegalencephaly, and ocular anomalies. She carried a mosaic gain of function mTOR p.Glu2419Lys variant in 41% of cells on skin DNA. Analysis of her dermal fibroblasts showed upregulation of mTOR signaling pathway. Because of an increasing frequency in seizures, and after approval of ethic committee, everolimus was initiated at 5 mg/day, and progressively increased up to 12.5 mg/day. Treatment was closely monitored, including with neuropsychological (Tremblay Scale for intellectual disabilities) and electroencephalographic assessment.

Results: After 6 months of treatment, and whereas blood concentration of everolimus was within the expected therapeutic range, no clinical efficacy was objectified; with no decrease in seizure frequency (120 seizures/month and 164 seizures/month at the baseline and after 6 months of treatment respectively). Brain MRI reevaluation revealed a frontal cortical dysplasia.

Conclusion: Inhibition of mTOR activation pathways with everolimus didn't induce clinical benefit in this case. The presence of frontal cortical dysplasia may explain therapeutic failure. Further observations are needed before drawing formal conclusions.

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E-P11.57
Autosomal Recessive Syngnathia Family

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Syngnathia refers to congenital fusion of the maxilla and mandible. The fusion can be classified depending on the nature of the connecting tissue as either fibrous or bony

fusion. The congenital fusion of the maxilla and mandible is a rare anomaly which is usually diagnosed after birth when it is discovered that the child is unable to open his mouth. Congenital synostosis of the mandible and maxilla is even less common than synechiae, with only 25 cases reported in the literature. Affected families reported in literatures, suggested autosomal dominant inheritance. A 7 months girl was referred to our center. Parents were first cousins. The clinical examination of the child on admission to the neonatal department reveal a reactive newborn with birth weight at 3400 g, a good sucking reflex and limited mouth opening with microretrognathia. Physical examination revealed microstomia, underdeveloped mandible, and inability to open the jaws. Her ex brother was also had same clinical features. As we know autosomal recessive syngnathia is not yet described in the literature. After some researches, we hope to find the possible gene or genes which causes this phenotype.

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E-P11.58

Different molecular approaches ministered to confirm diagnosis of thrombocytopenia-absent radius syndrome (TAR)

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Introduction: Thrombocytopenia-absent radius syndrome is a rare congenital condition characterized by bilateral absence of radius bone in each forearm and platelet deficiency. Most cases result from combination of deletion up to 400 kb in 1q21.1 chromosome region and point mutation in *RBM8A* gene. Although phenotypically readily diagnosed after birth, the genetic determination of particular SNPs in TAR syndrome harbors valuable information to evaluate disease severity and treatment management.

Aim: To refer a case report of male proband with TAR phenotype. Using different approaches, clinical exome analysis and chromosomal microarray as well as NGS whole genome analysis confirmed diagnosis of TAR syndrome.

Material and Methods: Clinical exome analysis was performed on MiSeq. Primary, vcf data was analyzed using Ingenuity Variant Analysis software. BAM data of whole *RBM8A* including intronic region was scrutinized on IGV Viewer. Additional CNV detection was provided by aCGH and low coverage whole genome sequencing (using GenomeScreen application). Afterwards, parental segregation analysis for carrier status was performed by Sanger sequencing and low coverage whole genome sequencing.

Results: We identified intronic variant in *RMB8A* (chr1: 145507765G>C, c.67+32G>C) annotated by ClinVar as pathogenic. Subsequently, 330 kb deletion in 1q21.1 region including *RBM8A* gene was detected by aCGH and GenomeScreen analysis confirming TAR syndrome diagnosis on molecular genetic level. Parental segregation analysis confirmed causality of identified candidate variants.

Conclusion: Combination of several approaches ministered to confirm TAR syndrome in a male proband. Results from different sources provide valuable information about genotype-phenotype correlation which is useful for future parental planning management.

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E-P11.59

MLPA as a practical and inexpensive first approach in a child with minor dysmorphisms, repetition pneumonia and malformation of great vessels revealed a complex familial partial *TBX1* gene exon loss

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Introduction: DiGeorge (OMIM#188400) and Velocardiofacial (OMIM#192430) Syndromes (DG/VCFS) represent genomic disorders (microdeletions of ~3Mb) in 22q11.2, characterized by facial dysmorphisms, heart defects and learning difficulties. Patients presenting with less distinctive traits should, however, be investigated for the presence of 22q11.2 microdeletions.

Materials and Methods: Multiplex ligation-dependent probe amplification (MLPA) targeted approach (Salsa® MLPA® kit P250-B2 DiGeorge Syndrome) was used for the detection of microdeletions and/or microduplications in one male patient aged 2 with minor dysmorphisms, repetition pneumonia and great vessels malformation and in his parents and maternal grandparents.

Results: The patient revealed both loss of amplification of *CLTCL1, HIRA, CDC45, CLDN5, GP1BB, TBX1-exon 7, TXNRD2, DGCR8* genes and no amplification of exon 2 in *TBX1* gene, corresponding to a microdeletion of ±2Mb in 22q11.2; his mother showed loss of amplification of exon 2 in *TBX1* gene and the father had loss of

amplification of *CLTCL1, HIRA, CDC45, CLDN5, GP1BB, TBX1, TXNRD2, DGCR8* genes. The maternal grandmother also presented with the loss of amplification of exon 2 in *TBX1* gene. Thus the proband's condition was proved to be inherited from alterations in both parents, resulting in homozygous absencer of exon 2 probe in *TBX* gene.

Conclusions: This is a rare case comprising *TBX1* gene-exon 2, resulting from genomic alterations in both parents. It is known that *TBX1* normal expression is important for normal heart development and its over/under expression may contribute to DG/VCFS. Future *TBX1* sequencing will provide a more accurate understanding of this situation and better counselling for this patient.

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E-P11.60
Atypical phenotype associated with a new *TCF4* variant: Call for additional cases

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Introduction: Haploinsufficiency of the basic helix-loop-helix (bHLH) transcription factor *TCF4* has been demonstrated to cause Pitt-Hopkins syndrome, associating mental retardation, wide mouth, distinctive facial features, intermittent hyperventilation followed by apnea. Pathogenic missense variants are primarily clustered within the C-terminal HLH domain required for dimerization and DNA binding. Although variants located elsewhere may be associated with mild non-syndromic intellectual disability, yet no variants have been reported to be associated with a malformative syndrome with normal cognitive development.

Methods: Here we report the case of an 11-year-old girl with a *TCF4* missense variant, facial dysostosis and normal cognitive functions.

Results: She was referred during the neonatal period for severe dysmorphism and intra-uterine growth retardation. She presented a square and asymmetric face, wide forehead, ptosis, depressed nasal bridge and ridge with hypoplastic alae nasi, microstomia, microretrognatism, and severely hypoplastic ears. Limb anomalies included fifth finger nail hypoplasia, fourth and fifth toes clinodactyly, low set fifth toe. She had lachrymal duct obstruction, responsible for chronic conjunctivitis. She had language delay, partially caused by conductive hearing impairment and facial dysostosis. At last evaluation, growth rate remained under the third percentile, although GH supplementation, to counterbalance moderate growth hormone deficiency, gave satisfactory results. Array CGH was negative. Trio exome sequencing allowed the identification of a de novo missense variant in *TCF4* (NM_001083962.1:c.1781T>A; p.(Met594Lys)) affecting an ultra-conserved amino acid within the HLH domain.

Discussion: If confirmed by additional patients, we may have identified a new clinical entity associated with *TCF4* variation.

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E-P11.61
Terminal 4q deletion: a new case report and review of the literature

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Terminal deletions of chromosome 4q are a rare event with approximate incidence 1/100,000. Chromosome 4q deletion syndrome is characterize by intellectual disability, craniofacial dysmorphism, rotated or low-set ears, cleft palate, micrognathia, congenital heart defects, craniofacial, skeletal and digital abnormalities, and occasionally autism spectrum disorder, behavioral disorders, and developmental delay.

Herein we report on a six month old boy with moderate dysmorphic features, congenital heart disease, developmental delay, hypospadias and a de novo deletion in 4q35.1–35.2 region, about 1.2 Mb, discovered on SNP and CNV array. The deletion was confirmed with subtelomeric and terminal 4q FISH probes. The kariotype was normal. The proband's phenotype was compared with previously reported cases with similar deletion in order to attempt genotype-phenotype correlation. We delineate intervals with great phenotypic overlap, particularly for congenital heart disease, developmental delay and possible autism spectrum disorder.

A. Mitroi: None. **M. Aschie:** None. **C. Brinzan:** None. **G. Cozaru:** None.

E-P11.62
The use of full-exsom sequencing as a modern method for verifying of TRAPS syndrome

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TRAPS syndrome (receptor-associated periodic syndrome) refers to genetically determined states that manifest with fever and clinical signs typical of rheumatic or autoimmune diseases in the absence of autoimmune or infectious causes. It occurs as a result of mutations in the TNFRSF1A gene on the short shoulder 12 chromosomes, which encodes one of the pro-inflammatory cytokine receptors. It is inherited as an autosomal-dominant disease. For the purpose of verifying the diagnosis, a molecular analysis of the TNFRSF1A gene is performed. We present the case of diagnosis of TRAPS syndrome in a patient O., 2012 of birth. At the age of 5 years the child got the consultation on genetics. He has got 2 healthy elder brothers. Heredity through the mother and father is not burdened. Harmful habits are denied by parents. For the first time episodes of febrile fever have appeared when the boy was 2.5 years old, after surgery for the inguinal killa. It lasted 3–7 days, it was repeated in 3–5 weeks, accompanied by abdominal pains. There was a significant increase in ESR. After the exclusion of oncohematological disease, nonspecific colitis, systemic rheumatologic and any infectious diseases, the autoimmune disease was suspected. Subsequent examinations made it possible to exclude hyperimmunoglobulin-D syndrome, PFAPA syndrome. By automatic sequencing, a partial analysis of the TNFRSF1 gene revealed a substitution in the 4th exon of 3449T> G: p.C117G, which allowed verifying the diagnosis of TRAPS syndrome. Application of sequencing technology of the new generation, allows verifying the diagnosis of complicated diagnostic cases.

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E-P11.63
TRIGONOCEPHALY ASSOCIATED WITH CHROMOSOMAL ABNORMALITIES

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Introduction: Trigonocephaly is a congenital condition involving premature fusion of the metopic suture and is associated with a risk of cerebral compression and several craniofacial morphological deformations. Trigonocephaly may be syndromic, involving other abnormalities, or isolated. Here, we report the clinical course of trigonocephaly associated with chromosomal abnormalities.

Cases: We report five cases. All cases were females, aged 1–6 years. Four patients exhibited chromosome 9p deletion syndrome and one had Jacobsen syndrome, known as 11q terminal deletion disorder. Three patients were suspected of chromosome 9p deletion syndrome at 4-month check-up because of head and neck instability, hypotonia, and frontal bossing. The others were diagnosed during the neonatal period. Three patients showed typical trigonocephaly and therefore underwent cranioplasty at 5 months of age, 3 years old and 4 years old, respectively. All patients who underwent surgery achieved satisfactory cosmesis. The infant with signs of cerebral compression experienced improvement in symptoms and could eventually develop speech and language post-surgery. Two patients exhibited isolated projection of the metopic suture. One was kept under observation and the other was referred for cranial remodeling because of positional plagiocephaly.

Conclusions: Patients exhibiting symptoms such as trigonocephaly, developmental delay, and hypotonia should be carefully examined at the 4-month check-up because such symptoms are signs of chromosomal abnormalities. Patients who underwent cranioplasty for trigonocephaly achieved satisfactory cosmesis, and one exhibited speech and language development post-surgery. Cranioplasty have a potential to improve symptoms also in patients with chromosomal abnormalities.

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E-P11.64
Partial Trisomy 9 - a Case Report

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Trisomy 9p, also called Rethoré Syndrome, is a rare chromosomal disorder caused by a duplication of the short arm of chromosome 9. The syndrome is characterized by distinct facial features, anomalies of the hand and feet, growth retardation, micro- and/or brachycephaly, a short and wide neck, scoliosis, and a variable degree of intellectual disability. We present a 24-year old female patient with developmental delay, dysmorphic facial features, scoliosis and abnormalities of the hands and feet. As a first diagnostic approach, we performed chromosome analysis, which identified the presence of an additional short arm of Chromosome 9 leading to the diagnosis of Trisomy 9p. Subsequent FISH and CGH array analysis verified the diagnosis of Trisomy 9p, but did not identify any additional chromosomal abnormalities. Next, we performed analysis on the patient`s mother, who was identified to be the carrier of a balanced translocation between chromosome 9 and 14. Hence, we concluded that our patient has inherited the additional short arm of chromosome 9 from her mother. X-ray imaging of our patient revealed a hypoplasia of the middle phalanx of the little fingers, a typical feature of Trisomy 9p patients. Additionally, a bipartite of the distal phalanx of both thumbs was identified in our patient. This rare skeletal abnormality, to our knowledge, has not been previously described in patients with Trisomy 9p. We conclude that skeletal abnormalities may be of a broader spectrum than previously thought in patients with Trisomy 9p and want to encourage further research in this area.

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E-P11.65
Spectrum of clinically diverse perinatal lethal phenotypes with urorectal septum malformation sequence

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Introduction: Urorectal septum malformation sequence (URSMS) is characterized by spectrum of anomalies of urogenital system, hindgut and perineum. It is hypothesized that URSMS is due to deficiency in caudal end of mesoderm or defect in cloacal endoderm, peri-cloacal mesenchyme, and genital ectoderm arising during early embryogenesis. Often, other malformations are also observed with URSMS and infrequently recurrence has been reported in families. A definitive etiology for URSMS is yet to be elucidated. Herein, we describe fetuses with URSMS with or without other malformations.

Materials and Methods: We reviewed fetuses with URSMS in referrals for perinatal autopsy over a period of three years. Literature was critically reviewed for syndromes and recurrent phenotypes with URSMS. Genomic evaluation (genomic sequencing) is underway for identification of a possible genomic etiology.

Results: Ten (10/215, 4.6%) fetuses had URSMS. Eight fetuses had complete URSMS. Partial and intermediate type of URSMS were noted in one fetus each. Nine fetuses had associated malformations of other system and are as follows: cerebral ventriculomegaly; right aortic arch with double outlet right ventricle; microcephaly, dysmorphism and pterygia; ventricular septal defect, radial ray anomaly and facial dysmorphism; thoraco-abdominoschisis, scoliosis and limb defects; myelomeningocele and vertebral segmentation defects; lumbosacral spina bifida and fused iliac bones; omphalocele and scoliosis; and occipital encephalocele. Commonly observed anomalies affected kidneys, neural tube, feet and heart. Together we report six recurrent and three newly described phenotypes comprising URSMS.

Conclusion: Several syndromes and recurrent phenotypes with URSMS remain uncharacterized. Further genomic and epidemiological investigations might identify unifying genomic bases.

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E-P11.66
The novel versican p.H2665L homozygous variation is associated with absence of cementum, early teeth loss and recessive Wagner vitreoretinopathy

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We studied an inbred family of Pakistani origin in which two first-cousin born brothers are affected by early teeth loss and absence of cementum formation. Whole exome sequencing revealed a novel p.H2665L homozygous sequence variant (MAF = 0) in the versican (*VCAN)* gene; the variation lies in the chondroitin sulfate attachment region β domain of the protein thus affecting both V0 and V1 *VCAN* isoforms. Teeth morphology showed dysplastic dentin with absence of predentin layer and calcospherites leading to a complete lack of cementum formation. Western blot analysis revealed a strong reduction of both V0 and V1 *VCAN* isoforms in proliferating cell lysates of patients' fibroblasts, with the V0 isoform almost absent. Dominant splicing mutations in *VCAN* are known to cause Wagner syndrome or vitreoretinopathy. Early signs of vitreoretinopathy were found in the elder brother while the parents were negative. Our studies support that the homozygous recessive p.H2665L missense sequence variant impairs the normal morphology of the teeth roots via loss of cementum synthesis, causing a novel *VCAN* phenotype but is also associated with early onset, recessive, Wagner syndrome, thus expanding both the phenotype mutation scenario and the inheritance mode of *VCAN* mutations.

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E-P11.67

Wiedemann-Steiner Syndrome with colobomas: expanding the phenotype and exploring overlap with other methyltransferase-related disorders

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Introduction: KMT2A is a methyltransferase involved in histone modification. Mutations in this gene cause Wiedemann-Steiner Syndrome, a disorder characterized by intellectual disability, short stature, and hypertrichosis cubiti. Colobomas have not been previously reported in WSS, however they are described as a feature in Kabuki Syndrome (KS). KS presents with characteristic facial features, skeletal abnormalities, intellectual disability, short stature, hirsutism, and ocular anomalies including colobomas. It is caused by mutations in KMT2D, and both KMT2A and KMT2D are in the mixed leukemia lineage (MLL) family of proteins. Both have histone lysine methyltransferase activity and are involved in developmental and hematopoetic gene regulation.

Case: Two brothers presented with colobomas, hirsutism, intellectual disability, dysmorphisms, and short stature. They were found to have c.11342_11345dupTTAA mutations in KMT2A. Their father, who had similar but milder features without colobomas, also carried the same mutation.

Conclusion: This case expands the known phenotypic spectrum of WSS to involve decreased expressivity and include colobomas, suggesting clinical overlap with other methyltransferase disorders. It highlights how next generation based genetic testing is helping us understand broader phenotypes of known genetic disorders and helping draw linkages between previously distinct disorders. This information will become even more important as we move into the age of molecular-based therapies.

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Diagnose Williams syndrome by FISH technique in Vietnam national childrens hospital

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Williams syndrome (WS) is a developmental disorder that affects many parts of the body. The most common symptoms of Williams syndrome are heart defects, unusual facial features, overfriendliness and behavioral problems. A form of cardiovascular disease called supravalvular aortic stenosis occurs frequently in people with Williams syndrome. Young children with Williams syndrome have distinctive facial features including a broad forehead, a short nose with a broad tip, full cheeks, and a wide mouth with

full lips. Many affected people have dental problems such as teeth that are small, widely spaced, crooked, or missing. In older children and adults, the face appears longer and more gaunt. Williams syndrome is caused by the deletion on a long arm of chromosome 7 (7q11.23) which includes 26 to 28 genes. WS affects an estimated 1 in 7,500 to 10,000 people at birth. In this study, the deletion at 7q11.23 was detected by FISH technique. We investigated 17 patients who were suspected WS in Vietnam National children's Hospital from august 2016 to august 2018. Results indicated that 10 patients (59%) have deletion at 7q11.23 to diagnose WS. They aged from 20 days to 22 months. All of them have supravalvular aortic stenosis. These results suggest that FISH technique is accurate, fast method and suitable to diagnose WS.

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E-P11.70
Wolf-Hirschhorn syndrome: clinical and genetic data analysis of Lithuanian patients

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Background: Wolf-Hirschhorn syndrome (WHS) is characterized by typical craniofacial features, growth deficiency, developmental delay/intellectual disability (DD/ID) of variable degree and other congenital malformations.

Methods: A cohort of 8 unrelated WHS patients who referred to the Centre for Medical Genetics was included into retrospective analysis in order to evaluate and compare clinical and genetic data. Genetic diagnosis has been established using conventional karyotyping, FISH or CMA. Clinical data of one patient was unavailable and was not included into clinical evaluation analysis.

Results: During the period of 2008 to 2018–8 patients were diagnosed with WHS. CMA was performed for 6 of them, and the size of deletion ranged from 3 Mb to 21.78 Mb. The age at time of diagnosis ranged from 4 months to 6 years. All patients represented the core clinical features—specific facial features, DD/ID, prenatal/postnatal growth retardation as well as feeding difficulties. Seizures were reported in 6/7 patients and hypotonia in 5/7 patients. Also 4/7 patients had hearing loss and ophthalmological problems—strabismus or hypermetropia. Congenital heart defects were observed in 6/7 patients, kidney anomalies in 3/7 patients and *corpus callosum* hypoplasia was detected in 2/7 patients. No other major internal organ malformations were reported.

Conclusion: WHS is well defined and our patients' clinical features overlap with phenotypes described in the literature. However, variable degree of clinical manifestation may complicate and delay establishing the diagnosis. The possibility of using microarrays has provided an increase in making an accurate genetic diagnosis.

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E-P11.71
Pathogenic variants in the gene encoding the cyclin dependent kinase 10 (CDK10) cause a wide spectrum of ciliopathy features

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Genetic defects in primay cilia formation, maintenance, or function underlie a wide array of ciliopathies in human including craniofacial, brain and heart malformations, retinal and hearing defect, polycystic kidney and recurrent respiratory infections. The cyclin-dependent kinase 10 gene (*CDK10*; OMIM:603464) regulates ciliogenesis and primary cilium elongation. Pathogenic variants in this gene have been associated with Al Kaissi syndrome (OMIM: 617694), an autosomal recessive developmental disorder characterized by growth retardation, spine malformation, particularly of the cervical spine, dysmorphic facial features and delayed psychomotor development. Herein, we report two male patients (2 and 8 years old; patient A and B, respectively) with shared symptoms including failure to thrive, global developmental delay, short stature and facial dysmorphism. Additionally patient A presented with recurrent respiratory infections, multicystic renal dysplasia and hypotonia. Interestingly the described facial abnormality for both patients partially mimics clinical presentation of Noonan Syndrome. Whole exome sequencing analysis revealed novel homozygous potential loss of function variants in the *CDK10* gene [Patient A: c.507dupG p.(Leu170Alafs*39); Patient B: c.985+6T>C p.(?)].

Although described in mouse model, to the best of our knowledge we are presenting here the first human record of multicystic renal dysplasia and recurrent respiratory infections, due to *CDK10* variations. Our findings underline the importance of the *CDK10* gene in the ciliogenesis process and further expand the phenotypic spectrum associated with a defect in this gene.

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E-P12
Cancer genetics

E-P12.01
No associations between rs4245739 and acute myeloid leukemia susceptibility

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Introduction: The single nucleotide polymorphism (SNP 34091A>C, rs4245739) of Mouse Double Minute 4 (MDM4) gene affect the MDM4 mRNA and protein level. In this way the mentioned SNP was reported to be associated with increased risk for several types of cancer (with conflicting evidence between different types of cancer). The aim of our study was to investigate if rs4245739 represent a risk factor for acute myeloid leukemia (AML).

Materials and Methods: In this case-control study a number of 705 subjects were enrolled, 403 healthy persons and 302 AML patients with approximatively equal distribution between the groups for sex and age. Tetra-ARMS PCR technique using specific primers was used for genotyping.

Results: The wild type genotype was found on 162 patients and 206 controls. The heterozygous genotype on 88 patients and 116 controls while the homozygous genotype with the variant allele on 52 patients and 81 controls. No statistical differences were observed for genotypes and alleles (dominant and recessive model) ($p>0.05$) between the groups. Similarly, no difference was observed between male and female patients.

Conclusions: Based on our results, MDM4 SNP 34091 A>C (rs4245739) is not a risk factor for AML development. Future studies are needed in order to investigate different SNP (or gene-gene) interaction.

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E-P12.02
Several cases of pancreatic and ovarian cancers in families with a mutation in the ATM gene. Is ATM an underrated cancer susceptibility gene?

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The use of multigenic panels in the diagnosis of hereditary cancers has become common in cancer genetics. In our Center, this panel contains 35 genes mostly implicated in breast, ovarian, colon, pancreatic and skin cancer.

From March 2016 to March 2019, we tested around 4370 patients for hereditary predisposition to cancer using Sophia Genetics' Hereditary Cancer Solution panel. 48 patients were carriers of a pathogenic variant of the ATM gene.

We found that in several families there were cases of ovarian cancer and pancreatic cancer (at least 20%).

These patients have a history of cancer, without telangiectasic ataxia.

It is currently known at present time that a mutation in the ATM gene induces an increased risk for breast cancer.

The increased risk for ovarian and pancreatic cancer, although being suggested and discussed, has not been clearly proven.

With limited knowledge on cancer risk at these specific sites, a careful genetic counseling was given during oncogenetic consultation. Patient management and genetic counseling took in account personal and family histories of cancer.

The objectives of this study were to highlight the cases of ovarian and pancreatic cancer in families with a mutation in the ATM gene, and compare these occurrence with the ones observed in families with a mutation in another gene or without any mutation found.

With these data we intend to question the risks of cancer associated with ATM, as well as a suggestion to reconsider guidelines established for the management of patients with a mutation in this gene.

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E-P12.05

Production recombinant Herceptin in prokaryotic system (E.*coli*) and evaluation its effect on breast cancer cell line

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Introduction: Breast cancer is one of the most common types of cancer among women, with a prevalence of about one million a year, causing a 7% mortality rate due to cancer. HER-2 molecule as the receptor of tyrosine kinase from the family of epithermal growth factor is a major cause of cancer. Herceptin works by attaching itself to the HER2 receptors on the surface of breast cancer cells and blocking them from receiving growth signals. By blocking the signals, Herceptin can slow or stop the growth of the breast cancer. The aim of this study was to subcloning of *Herceptin* gene, expression in the prokaryotic system (*E. coli*) and produce Herceptin recombinant protein for use in the diagnosis of breast cancer.

Materials and Methods: Herceptin gene which was subcloned in pET28a vector had plasmid purified and became digested, then, after protein expression and confirming with Western blotting, transferred to E. coli BL21DE3 by a heat shock technique.

Results: Protein expression was induced by IPTG and optimized expression of recombinant protein, purification and evaluation by western blotting method was established. At the end, the effect of recombinant Herceptin protein on the cancer cell line was investigated by MTT.

Conclusions: Levels of antigen related to breast cancer can be used as a predictor of the disease. Herceptin can be useful both in treating and detecting such a cancer.

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E-P12.06

Next generation sequencing for analysis of the spectrum of somatic mutations in bulgarian breast cancer patients

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Introduction: Breast cancer is the most commonly diagnosed malignancy and the most frequent cause of death in women due to cancer in Bulgaria. The increased incidence of breast cancer determines the need for improving approaches to diagnosis, treatment and follow —up of the disease. Next Generation Sequencing (NGS) of cancer-associated gene panels can detect somatic mutations in tumors more sensitive and specific than Sanger sequencing.

Materials and methods: In the current study we included a group of 91 Bulgarian patients diagnosed with breast cancer. DNA was isolated from fresh frozen tumor tissues. We have analyzed all samples for the spectrum of somatic alterations in a panel of 48 cancer related genes by NGS.

Results: Pathogenic somatic mutations were found in 65.9% of the patient samples. They are spread in 35% of the genes included in the panel. Most frequently mutated genes were PIK3CA, ERBB2, APC, ATM and TP53 which differs from the most common mutated genes in breast cancer according to COSMIC database. From all mutations found, p.Thr1743LysfsTer13 in ATM gene (12% of patients) and p.Thr759Pro in ERBB2 gene (12% of patients) were the most common.

Conclusion: NGS sequencing of cancer related genes became fast and high sensitive method for somatic mutation analysis of heterogeneous tumors. The established profile of the somatic mutations in PIK3CA, ERBB2, APC, ATM and TP53 in the study group of tumors in Bulgarian breast cancer patients differs from that in the databases, which confirms the need for more research in this direction.

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E-P12.07

PKI-402 as a radiosentisizer in breast cancer cell model

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Introduction: Cancer stem cell (CSC) population becomes dominant following therapeutic ionizing radiation (IR) exposure accompanied by the activation of the PI3K pathway. A potent dual pan-PI3K/mTOR inhibitor PKI-402 was evaluated in different breast cancer subtypes for radio sensitizing activity.

Material and method: In our study, the radio sensitizing activity of PKI-402 was evaluated in breast cancer cell lines MCF-7 and MDA-MB-231 and breast CSC. Cytotoxicity, apoptosis, DNA double-strand break (γ-H2AX), clonogenic survival and phosphorylation assays were performed and compared with normal breast epithelial cells (MCF-10A).

Results: IC_{50} values of PKI-402 at 48th hours were determined as 109 nM, 2.44 μM, 5.94 μM and 142 nM for MCF-7, MDA-MB-231, BCSC and MCF-10A, respectively. Significant differences in survival percentage were observed for MCF-7 (19.93 and 15.61%) and BCSC (31.93 and 28.04%) as compared to IR (4 Gy) and PKI+IR (4 Gy) treatment. Apoptosis induction was determined in MCF-7, BCSC and MCF-10A cell lines. There was a significant increase in the double strand breaks in MCF-7 and MDA-MB-231 cell lines in both groups (IR and PKI+IR), but no significant changes in MCF-10A and BCSC lines. For all cancer cell lines there was a significant decrease in the phosphorylated proteins indicating the PI3K/mTOR pathway suppression, whereas for MCF-10A decrease was observed only in the 4E-BP1.

Conclusion: PKI-402 was found to be effective as a radiosensitizer in breast cancer cell models. The findings should be confirmed in further experiments.

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E-P12.11
Fibroblast growth factor receptor 4 (FGFR4) variant p. Gly388Arg in Czech children carcinoma patients

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Variant p.Gly388Arg in the gene encoding for FGFR4 is present at significantly higher frequency in cancer patients with aggressive disease progression and therefore represents a gene alteration that predisposes the carrier to poor clinical outcome. To determine the FGFR4 polymorphism status, and the correlation with clinicopathological features in Czech children carcinoma patients, we performed genotyping of Gly388Arg *FGFR4* variant.

Method: This was a retrospective study which included 100 children advanced carcinoma patients who had undergone surgery at the Clinic of children's oncology of the University Hospital Brno. In cooperation with TIBMolBiol, we designed LightSNiP assay (LightCycler Technology with SimpleProbe®) to detect the Gly388Arg *FGFR4* variant. The genotyping PCR reactions were performed using LightCycler® 480 Instrument II and LightCycler® FastStart DNA Master HybProbe (Roche Diagnostics, Germany).

Results: From amplification and detection with LightSNiP probe by melting curve analysis, it was possible to obtain a visual discrimination of Gly388 and Arg388 alleles in the homozygous and heterozygous status. In 100 children carcinoma patients, 59 (18/31% deceased) were Arg388 *FGFR4* and 41 (6/15% deceased) were 388Gly *FGFR4*.

Conclusion: We investigated the prognostic significance Arg388 *FGFR4* in children carcinoma patients using the melting curve analysis, which is a sensitive methodology to screen for sequence variants in clinical samples. In summary, *FGFR4* Arg388 was significantly associated with survival. Thus, the role of *FGFR4* polymorphism is a prognostic marker for children advanced carcinoma patients. Our results may lead to improved prediction of clinical prognosis as well as novel therapeutic strategies that target the FGFR4 signaling pathway.

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E-P12.12
An integrative model of influences on cancer prevention, detection, and treatment

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Our scientific and clinical understanding of cancer is changing. Advances in technology coupled with new discoveries in cancer biology are forcing a broadened perspective and with that, recognition of a more inclusive model of cancer's influencing factors. We present a multidimensional dynamic model of the interactions of genomic, epigenomic, and environmental factors that can provide research pathways for future studies. These are predicted to stimulate the development of comprehensive cancer algorithms and the evolution of universal precision medicine. The current inadequacy of human genomic databases on African-descended human groups and their cancer tumor genomics are discussed and the efforts at

Howard University to ameliorate these deficiencies through the development of comprehensive genomic databases. The importance of systematically addressing environmental catalysts for cancer including bioactive dietary factors, the body's commensal microbiota, and the role of immunotherapy in cancer control are discussed. The need for population-and cancer-specific biomarkers to be identified and algorithms developed is highlighted and the role for large-scale studies of African-descended groups emphasized.

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E-P12.15
Prevalence of human cytomegalovirus infection among Bulgarian patients with brain tumours

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Brain tumours are among "hard-to treat" world malignancy. They are characterized by rapid progression, infiltration and therapeutic resistance. Recent studies reveal the putative oncomodulatory role of Human cytomegalovirus (HCMV) in the development of these tumours. The presence of HCMV has been shown in a number of high-grade gliomas and brain metastases. The prevalence and role of HCMV were analysed among Bulgarian patients with primary and secondary brain tumours. Genomic DNA from 10 patients with primary Glioblastoma multiforme (GBM) and 10 secondary tumours was isolated from whole blood and tumour tissue by commercial kit. The samples were analysed by PCR for the presence of HCMV DNA. Our study shows the presence of viral DNA in around 10% of the tumour tissues. Interestingly, in one case, HCMV DNA was found in the tumour tissue of recurrent GBM but not in whole blood pointing to the reactivation of the virus after the treatment. This is a pilot study analysing CMV infection in a small cohort of Bulgarian patients with brain tumours. The oncomodulatory effect of HCMV may dysregulate critical signalling pathways and contribute to malignant transformation. Our findings of secondary reactivation of HCMV in recurrent brain tumour tissue is in concordance with the recent data for radio/chemotherapy-induced cytomegalovirus (CMV) reactivation. More studies are needed to evaluate the role of HCMV infection for brain tumour management and therapy strategies. The study was supported by Medical University Sofia, Grant number D-118/2018

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E-P12.16
Lynch syndrome family case resulting from secondary finding

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Introduction: Lynch syndrome is a hereditary cancer predisposing syndrome with autosomal dominant inheritance and incomplete penetrance caused by the germline mutation in one of the DNA mismatch repair genes. Carriers of the mutation are at an increased lifetime risk for colorectal, endometrial, ovarian and other more rare types of cancer. The most commonly mutated genes are MLH1 and MSH2 (90%), however germline pathogenic variants in MSH6 account for 10–20% of Lynch syndrome. New cases are diagnosed based on Amsterdam criteria and Bethesda guidelines, but it is estimated that many cases remain undetected.

Material and Methods: Here we describe a 19 years old man, who was tested for early onset inflammatory bowel disease, using Trusight ONE gene panel. Additional analysis of secondary findings in relevant genes was performed.

Results: We identified an increased risk for Crohn´s disease and we also found secondary findings including two splicing junction variants in MLH1, heterozygous ultra-rare missense variant in MSH6 and heterozygous rare BRCA2 missense variant, both classified as likely pathogenic variants. Result of colonoscopy was negative. Father and grandfather are carriers of both variants (BRCA2 and MSH6). Colonoscopy of grandfather shows benign and borderline polyps.

Conclusions: Slovakia is one of the leading countries in the incidence of colorectal carcinoma. We postulate hypothesis that Slovakia is a country with more abundant pathogenic variants in mismatch repair genes and thus preventive screening program should be offered.

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E-P12.17

The clinical phenotype of codon 790 RET Proto-oncogene mutations in Slovenian MEN 2 patients

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Introduction: RET mutations causing multiple endocrine neoplasia type 2 (MEN2) have been divided into different risk groups for medullary thyroid carcinoma (MTC), among them RET 790 mutation represents the only mutation with reduced penetrance. Exact genotype-phenotype correlation is important since genetic analysis usually dictate the timing for prophylactic thyroidectomy (1). RET 790 mutation represents the second most prevalent RET mutation in Slovenian cohort of MTC patients.

Patients and methods: We included 185 MTC patients for genetic counselling and for RET oncogene screening of exons 10, 11, 13, 14, 15 and 16. High Resolution Melting analysis (HRM) analysis and sequencing in both directions were used for mutational analysis.

Results: Mutations in the RET oncogene and VUS were found in 66 mutation carriers (34%) from 31 families. We detected 17 different types of mutations and VUS in the *RET* proto-oncogene. p. L790F mutation in exon 13 of RET gene was found in 19 patients from 5 families. Phenotypically, all but 2 cases had FMCT or HCC with 3 cases of concomitant PTC too.

Conclusions: Genetic testing of RET gene leads to the correct diagnosis of MTC patients and to regular surveillance and early treatment. RET 790 mutation considered as mutation with a non-aggressive form of MEN 2 in our cohort of MTC patients show more aggressive course as previously reported (1).

Reference

1. Bihan H, Murat A, Fysekidis M, et.a., The clinical spectrum of RET proto-oncogene mutations in codon 790. Eur J Endocrinol. 2013 Jul 29;169(3):271–6. doi: 10.1530/EJE-13–0050.

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E-P12.18

The association of TNF alpha -308 (rs1800629) and -238 (361525) polymorphisms with the risk and outcome in multiple myeloma patients treated with thalidomide and/or bortezomib

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Introduction: Multiple myeloma (MM) is a disorder characterized by activation of malignant B-cells. They produce tumor necrosis factor (TNF), which is a proinflammatory cytokine. There are two SNPs at the positions −308 and −238 of the 5′ promoter region of *TNF-alpha* gene. They might be associated with the level of TNF synthesis and MM aggressiveness.

Methods: A group of 100 MM patients and 100 healthy volunteers was genotyped. Both polymorphisms -308G>A and -238G>A were assessed by PCR-RFLP.

Results: Genotypic frequencies of -308G>A and -238G>A for healthy individuals and MM patients were balanced. The difference between allelic frequencies of studied polymorphisms in control and study groups were statistically insignificant. In the case of both polymorphisms GA and AA genotypes were analyzed together due to small number of AA. An association between the risk of MM and -238GA+AA genotypes was observed; $OR = 2.0$ (1.09–3.65); $p = 0.002$. After auto hematopoietic stem cell transplantation MM patients -308GG and -238GG showed longer OS when treated with thalidomide than with bortezomib or combination of both (56.8 vs. 12 months, $p < 0.001$ and 62.8 vs. 12 months, $p < 0.001$). Similar results were observed in PFS analysis. No association was found of TNF polymorphisms with the response rate or laboratory results.

Conclusions: The -238G>A polymorphism might be associated with the risk of MM development. MM patients with -308GG and -238GG genotypes showed better outcome (longer OS and PFS) when treated with thalidomide as compared with bortezomib. Further studies on a larger group of patients are necessary to confirm these results.

S. Zmorzyński: None. **S. Popek-Marciniec:** None. **A. Szudy-Szczyrek:** None. **M. Wojcierowska-Litwin:** None. **I. Korszeń-Pilecka:** None. **W. Styk:** None. **S. Chocholska:** None. **D. Koczkodaj:** None. **M. Hus:** None. **A. A. Filip:** None.

E-P12.20

Building High Quality NGS DNA Libraries from Ultra-small Fragments

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Researchers are extending the use of next generation DNA sequencing and library preparation kits to highly challenging sample types such as ancient DNA and FFPE purified DNA as well as very small chromatin immunoprecipitated (ChIP) samples and cell-free DNA. In this study we describe a modified, modern library prep method for the construction of high quality DNA fragment libraries from extremely challenging and small samples. Examples for the application of this technology include both synthetic libraries using various size dsDNA oligos (10 bp to 90 bp) to demonstrate protocol capabilities as well as experimental data with cell-free DNA and micrococcal nuclease-digested chromatin immunoprecipitated material.

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E-P12.21
Classification of novel variants in clinically important genes beyond hotspot codons

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Introduction: In a daily routine laboratory practice the variants outside the hotspots of clinically important genes (e.g. *BRAF*, *KRAS*, *NRAS*) are associated with challenges in classifying and reporting of their clinical relevance. Consequently, the use of standardized consensus classification, interpretation, and reporting is needed. Our aim is to present the adoption of recommended standards and guidelines for reporting tumor genotyping results in colorectal cancer, malignant melanoma and thyroid cancer.

Materials and methods: NGS library was prepared from 88 FFPE samples using TruSightTumor 15 (Illumina). The variant actionability was assessed according to Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer[1].

Results: 88 patients were screened for the presence of actionable mutations in genes *BRAF, KRAS*, and *NRAS*, among which 63 had colorectal cancer (CRC), 14 malignant melanoma and 11 thyroid cancer. Along with well-studied activating variants, we have discovered four variants which were not well-characterized in the literature or in public databases(Table 1).

Conclusions: Well-characterized activating mutations are easy to interpret in contrast to variants found in the vicinity of a hot-spot codon, without functional or clinical characterization. In order to characterize the variant's clinical actionability it is essential for the clinical laboratories and clinicians to report novel variants.

Table 1. Variants identified outside of well characterized mutational hotspot codons.

1.Li. et al. *J. Mol. Diagn.* **19**, 4–23 (2017).

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E-P12.25
SELINA - clinical trial on lowering the risk of malignancies by optimizing selenium levels in females from families with hereditary breast cancer

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Aim: Prospective observational studies showed that blood selenium (Se) levels associated with significantly lower risk of cancers can be identified in Polish females from families with hereditary breast cancers (HBC). For BRCA1 mutation carriers it is: 70–89 μg/l at age <50 yrs (OR~12) and 95–120 μg/l at age ≥50 yrs (OR~4). For females without detected BRCA1 mutation but from families with pedigree/clinical features of HBC it is 98–108 μg/l (OR~5).

The main goal of SELINA is validation of hypothesis that optimization of Se level by supplementation or diet changes can decrease the risk of malignancies in groups described above.

Method: 7000 females (including 1200 BRCA1 carriers) from families with HBC and deficiency or excess of Se will be recruited and randomly qualified to one of the following arms: "placebo", prospective observational, supplement (Sodium Selenite) or diet modification. Blood Se level will be systematically measured using ICP-MS and appropriately optimized. Follow-up will take 5 years.

Results: At present we are performing recruitment. It is planned to close it at the end of 2019.

Conclusion: SELINA is the first clinical trial aimed to decrease the risk of cancers by active control of blood selenium levels. All interested scientists/institutions are welcome for collaboration.

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J. Lubinski: None.

Gene	Variant (HGVSc)	Amino Acid Change (HGVSp)		VAF (%)	Tumor Type	times observed	Previously described in CRC	Clinical actionability[1]
BRAF	c.1781A>G	p.(Asp594Gly)	D594G	24.0, 50.0, 37.0	CRC	3	Yes (COSMIC 38x)	Tier II, level C
BRAF	c.1805C>T	p.(Ser602Phe)	S602F	5.6	CRC	1	Yes (COSMIC 1x)	Tier III, VUS
KRAS	c.349_351delinsTAT	p.(Lys117Tyr)	K117Y	34.0	CRC	1	No	Tier III, VUS
NRAS	c.37G>C	p.(Gly13Arg)	G13R	27.6	CRC	1	Yes (COSMIC 18x)	Tier II, level C

E-P12.27

The study of TP53 gene in patients with small cell lung cancer

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Lung cancer is one of the most common cancers and is the leading cause of cancer death worldwide. Small cell lung cancer (SCLC) is neuroendocrine tumor representing 10–15% of all lung carcinoma cases. SCLC exhibits aggressive behavior, rapid growth, and early development of metastases. A large number of genes involved in SCLC development. In up to 90% of all SCLCs cases TP53 gene coding for p53 transcription factor is mutated. We performed a search for mutations in 17q13.1 locus containing TP53 gene. Material and methods: study included 80 FFPE SCLC samples; method used was multiplex ligation-dependent probe amplification (MLPA). MLPA revealed 15 SCLC samples with heterozygous duplications. They were found in the 1st (11 samples), 2nd, 4th, 7th and 10th exons (one each) of TP53 gene. Most of the samples still have deletions in the 17p13 locus. The majority of them located in the 7th exon of MPUD1 and in the 1st exon of the ATP1B gene (five samples). Four samples have deletions in the 4–6th exons of TP53 gene, three samples—in the 2d exon of TP53 and 7th exon of ATP1B2, and one sample—in the 13th exon of CHEK2 gene. Besides, four samples revealed 1100delC mutation in CHEK2. This deletion is associated with breast, ovarian and prostate cancers in different populations, but never been described in SCLC yet. Our findings, in general, correspond with the literature results, showing high mutations rates of TP53 gene in SCLC. The study was supported by RFBR project №17-020115.

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E-P12.28

Developing solid tumor and hematological panels for a flexible and automated microfluidic workflow that improves efficiency of NGS library preparation for cancer research

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The promise of personalized medicine endeavors to apply insights from actionable mutation data toward individualized treatment strategies. Panels and workflows enabling rapid interrogation of multiple mutation types are desirable. We designed a targeted NGS library preparation (LP) panel that covers >230 kb of exon sequence from actionable mutations in 53 genes from solid tumors. We also designed an RNA fusion NGS LP panel detecting more than 1,000 unique fusion breakpoint events in >350 fusion gene pairs from both solid and hematologic tumors. Both panels utilize a highly multiplexed, nanoliter-scale PCR-based enrichment method performed on the automated Fluidigm Juno™ system, providing streamlined, cost-effective NGS LP. Amplicon LP of 1–6 unique panels can be processed simultaneously. Samples can be assayed with LP panels in groups of 8, enabling up to 48 samples per run. This workflow coupled with relevant panel content and performance offers a solution for investigators requiring economical NGS of FFPE and blood samples. Reference standards and clinical research FFPE samples were used to assess panel performance, including positive predictive agreement (PPA), positive predictive value (PPV), and limit of

detection. The DNA solid tumor panel uses 12.5 ng of input DNA, with PPA and PPV >97% for SNVs, indels, and CNVs with allele frequencies down to 5%. Amplicon uniformity (>0.2x and <5x of mean coverage) is >90%. From 10 ng of total RNA, PPA and PPV were >99% for the fusions tested and could be reliably detected with as few as 250 copies of target fusion events.

T.J. Goralski: A. Employment (full or part-time); Significant; Fluidigm. **M. Gonzales:** A. Employment (full or part-time); Modest; Fluidigm. **J. Perez:** A. Employment (full or part-time); Modest; Fluidigm. **J. Qin:** A. Employment (full or part-time); Modest; Fluidigm. **S. Chamnongpol:** A. Employment (full or part-time); Modest; Fluidigm. **D. Wang:** A. Employment (full or part-time); Modest; Fluidigm. **A. Lespagnol:** None. **J. Jasper:** A. Employment (full or part-time); Modest; Q2 Solutions|EA Genomics. **J. Brockman:** A. Employment (full or part-time); Modest; Fluidigm. **G. Harris:** A. Employment (full or part-time); Modest; Fluidigm. **J. Wang:** A. Employment (full or part-time); Modest; Fluidigm. **J. Alipaz:** A. Employment (full or part-time); Significant; Fluidigm. **C. Park:** A. Employment (full or part-time); Significant; Fluidigm. **J. Geis:** A. Employment (full or part-time); Significant; Fluidigm. **G. Sun:** A. Employment (full or part-time); Significant; Fluidigm. **B. Fowler:** A. Employment (full or part-time); Significant; Fluidigm. **C. Kabu:** A. Employment (full or part-time); Significant; Fluidigm. **D. King:** A. Employment (full or part-time); Significant; Fluidigm.

E-P12.29
Genomic Profiling of two distinct morphological portions of a Uveal Melanoma by Array-CGH

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Uveal melanoma (UM) is the most common primary intraocular tumour, with an incidence of approximately 5.1 cases per million, with a strong propensity for haematogenous spread. Survival is almost entirely dependent on whether patients develop liver metastases. Currently, the most common treatment options are radiation therapy or enucleation. There is a direct association between UM and poor prognosis chromosomal alterations like monosomy 3 and 8q gain. A wide range of techniques can be used in the UM genomic profiling: routine cytogenetic analysis, fluorescence *in situ* hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), array comparative genomic hybridization (aCGH) and gene expression analysis (GEA). We report the genomic profile of two distinct morphological portions of a UM in a male patient. The two samples from fresh tumour tissue (pigmented and non-pigmented) were taken for Array-CGH analysis following enucleation. DNA extraction, quantification and quality assessment, DNA labelling and hybridization were performed using Agilent SurePrint G3 CGH 4X180K platform. Tissue identity was determined by comparing 15 STR loci pattern of UM tissue with patient blood profile, using AmpFLSTR Identifiler Amplification kit. Array-CGH analysis identified in both tissues: monosomy 3, 8q gain combined with 8p loss (possible isochromosome 8q) and X monosomy (loss of chromosome Y). A 6q deletion was only observed in the pigmented sample, possibly representing a late event in tumorigenesis. In conclusion, Array-CGH has proved to be a powerful genomic tool to define chromosomal imbalances in UM patients but results can be conditioned by intratumoural heterogeneity and tissue sampling bias, compromising prognostication.

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E-P13
Basic mechanisms in molecular and cytogenetics

E-P13.01
From human cytogenetics to human chromosomics

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The designation "chromosomics" was introduced by Prof. Uwe Claussen (30. April 1945–20. July 2008) back in 2005. Chromosomics is about chromosomes, their three-dimensional positioning in the interphase nucleus, the consequences plasticity of chromosomal subregions and genes interacting have, the influence of these chromatin-modification-mediated on functions of cells and even individuals, as well as about evolution and disease. Here it will be highlighted how the growing insights into human chromosome structure finally led to a "chromosomic view" on the three-dimensional constitution and plasticity of genes in interphase nuclei. The progress achieved in recent years includes e.g. the detection of chromosome-chromosome-interactions which, if damaged lead to malfunctions and

diseases. However, chromosomics is not progressing in some parts at present, as research interest shifted from single cell to high throughput, genomic approaches. Chromosomics and its potential have been predicted correctly in 2005 by Uwe Claussen. Thus, at present reconsiderations of taking the chromosome and the single cell into human genetic research focus again, are urgently necessary.

T. Liehr: None.

E-P13.02
Cytogenetic abnormalities of T/NK cell lymphoma patients in Singapore

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Introduction: T/NK-cell lymphoma constitutes just 12% of non-Hodgkin lymphoma while the remaining are B-cell lymphoma. Their rarity has resulted in limited data on the recurring chromosomal alterations. Difficulties in precise classification due to the heterogeneity of morphological features have also confounded the data. Little is also known regrading predilection of the disease due to ethnicity.

Materials & Methods: A retrospective study was done from 2001–2018 on 200 patients with first diagnosis of T/NK-cell lymphoma. Their age ranged from 17–88 years (median age 55 years). Owing to morphological variations, a large number of cases could not be subtyped. 48-h and PHA-stimulated 72-h bone marrow cultures were established. Following slide-making, GTG-banding was performed for karyotyping.

Results: There were 146 Chinese (73%), 29 Malays (14.5%), 9 Indians (4.5%) and 16 other nationalities (8%). 172 patients (86%) had no pathological karyotypic abnormalities while 28 patients (14%) exhibited acquired chromosomal abnormalities. Recurrent numerical abnormalities included trisomies 1, 7 and 8, and complex karyotypes. The most frequent structural rearrangements involved deletions 4q, 6q, 7p, 7q, 9p, 10p, 14q and i(7q) and i(8q).

Conclusion: Only 14% of T/NK cell lymphoma patients exhibited chromosomal aberrations.

The occurrence of T/NK cell lymphoma in the Singapore population shows approximately the same distribution in terms of our ethnicity, suggesting that ethnicity may not be a factor although geographical variations have been reported. The 14% detection rate is unacceptably low. Granted that some abnormalities may be subtle, future work could involve Next Generation Sequencing technologies to increase the abnormality detection rate.

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E-P14
New diagnostic approaches - Technical aspects - Quality control

E-P14.01
A new diagnostic approach for Congenital Adrenal hyperplasia; measuring 17- Hydroxyprogesterone via biosensor

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A new diagnostic approach for Congenital Adrenal hyperplasia; measuring 17- Hydroxyprogesterone via biosensor

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Introduction: Measurement of 17-OHP is therefore valuable in the initial diagnosis of Congenital Adrenal Hyperplasia. The most prevalent form of the disorder is 21-hydroxylase (21-OH) deficiency which is the most frequent inborn metabolism error and 17-OHP is secreted in abundant excess. Methods for measuring 17-OHP procedures for are still suboptimal because of low specificity, particularly in premature infants. The aim of this study was to design an enzyme based biosensor for detection 17-OHP; a new alternative method for CAH diagnosis.

Materials and Methods: The measurements were performed using a gold electrode coated with Au-Poly HemaMac. UV immobilization performed with 17-OHP-peroxidase on the modified gold electrode.

Results: Optimization studies determine the most suitable working conditions for using the biosensor. Polymerization time was 2h, the enzyme concentration used 0.5mg/mL, temperature was 35 °C and pH was 6.5 with phosphate buffer. After the characterization studies of the biosensor the detection limit was 0.015 ng/mL-20 ng/mL, repeatability was 2.98±0.04.

Conclusions: The demonstrated new method for 17-OHP detection can be used in newborn screening as a point of care test, is useful and can be carry out rapidly in clinical diagnosis. Biosensors are reproducible, quick and results can be generated within a few minutes.

E. Yenilmez: None. **U. Kokbas:** None. **A. Tuli:** None.

E-P14.03
Quality control of DNA and RNA samples using the 4150 TapeStation platform

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Quality control (QC) of RNA and DNA samples is key for the success of any downstream experiment. Especially, Next Generation Sequencing (NGS) developed to a powerful tool in almost all genetic research and diagnostic areas. Since the downstream applications are often time-consuming and expensive, tight QC steps are required to avoid a "garbage in-garbage out" situation. The ideal QC solution is easy-to-use, economical and provides fast and unambiguous results also for very low concentrated samples. Nucleic acid quality assessment can be standardized using automated electrophoresis systems to ensure that samples are "fit for purpose". This poster exhibits the latest developments in nucleic acid sample QC and gives application examples—from gDNA to NGS libraries and RNA samples—evaluated with an Agilent 4150 TapeStation system. Quality scores enable impartial and user independent sample comparison and allow defining a quality threshold for specific types of samples and preparation. For the objective quality evaluation of gDNA and RNA, the quality scores DNA integrity number (DIN) for gDNA and the RNA integrity number equivalent (RIN^e) for RNA can be assessed providing numerical values from 1 (degraded) to 10 (intact) for classification of samples.

R. Nitsche: A. Employment (full or part-time); Significant; Agilent Technologies. **B. Strauch:** A. Employment (full or part-time); Significant; Agilent Technologies. **C. Lotter:** A. Employment (full or part-time); Significant; Agilent Technologies. **C. Voigt:** A. Employment (full or part-time); Significant; Alacris Theranostics.

E-P15
Personalized/predictive medicine - Pharmacogenomics

E-P15.04
Tramadol pharmacogenetics and tramadol-related adverse events in patients after breast cancer surgery

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Introduction: Breast cancer patients usually receive tramadol for pain treatment after surgery, however, some patients experience insufficient pain relief or adverse events when using standard therapeutic doses. Genetic variability of metabolizing enzymes or drug transporters may influence both efficacy and toxicity of tramadol. The aim of this study was to evaluate the association of genetic variability in tramadol pharmacokinetics pathway on short-term adverse events in breast cancer patients.

Materials and Methods: The study included 113 breast cancer patients receiving either 75 or 37.5 mg of tramadol for pain relief after breast cancer surgery as part of a randomized clinical trial KCT 04/2015-DORETAonko/si at Institute of Oncology Ljubljana. All patients were genotyped for 14 polymorphisms in *ABCB1*, *ABCC2*, *CYP2D6*, *SLC22A1* and *UGT2B7* genes and *CYP2D6* duplication and deletion. The association with treatment discontinuation or tramadol-related adverse events within four weeks after surgery was evaluated using logistic regression and Fisher's exact test.

Results: Carriers of two polymorphic *UGTB7* rs28365063 or *SLC22A1* rs628031 alleles were significantly more likely to discontinue tramadol treatment due to adverse events (OR = 10.83, 95% CI = 1.59–73.91, P = 0.015 and OR = 6.81, 95% CI = 1.42–32.53, P = 0.016, respectively), even after adjustment for tramadol dose (P = 0.025 and P = 0.044, respectively). Regarding specific adverse events, CYP2D6 poor metabolizers experienced less constipation (P = 0.013), while other genes were not significantly associated with any adverse events.

Conclusions: Genetic variability of genes involved in tramadol pharmacokinetics may be associated with adverse events in breast cancer patients, suggesting pharmacogenetic testing could enable a more personalized treatment.

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E-P15.06
Molecular determination of paternal RHD zygosity to predict risk of hemolytic disease of fetus and newborn

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Introduction: Hemolytic disease of the fetus and newborn (HDFN) results from sensitization of the mother's immune system to foreign antigens present on the red cells of the fetus. Determining the RHD zygosity of the father is valuable for the management of HDFN related to anti-D. For years in different districts of Albania during the transition period, the Rhesus blood group was not mandatory typing. Consequently, D alloimmunization in pregnancy still occurs. Here we report a robust clinical assay for RHD zygosity using molecular techniques.

Materials and methods: This study tested 54 paternal samples from respective alloimunized RhD negative pregnant women. Samples were tested using DiaClon monoclonal antibodies. Genomic DNA was extracted and PCR was performed using the PCR system and primers *rez*7 (nonspecific, 5 'of Rhesus box identity region) and *rnb*31 (specific for 3′ of downstream Rhesus box identity region). The PCR amplicons were digested with *PstI*. PCR-RFLP fragments were resolved using a 1% agarose gel. Bands were stained with EtBr and visualized by UV Imaging system.

Results: This PCR- RFLP method accurately determines the RHD-haplotype, characterizing our samples in: 73% (39 individuals) RHD + / RHD + homozygous, and 27% (15 individuals) RHD + / RHD—heterozygous. RHD zygosity in paternal samples will help predict the risk that a fetus will inherit RHD. Here a method for determination of the *RHD* zygosity status of the father in order to predict the hemolytic disease of the fetus and newborn was standardized.

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E-P16
Omics - Bioinformatics

E-P16.01
Structural evaluation of genetic variations in *CYP21A2* gene

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Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroidogenesis. Disorders in steroid21-hydroxylation account for over 95% of patients with CAH. Clinically, the 21-hydroxylase deficiency has been classified in a broad spectrum of clinical forms, ranging from severe or classical, to mild late onset or non-classical. Over the years, a large number of variants of the *CYP21A2* gene has been found in the regulatory, promoter, coding and intronic regions. Even though most patients carry *CYP21A1P*-derived mutations, an increasing number of novel and rare mutations in disease causing alleles were found in the last years. This work approaches the bioinformatic analysis of novel variants in the coding region of the *CYP21A2* gene. The variants were evaluated in three-dimensional *CYP21A2* structures, using program FoldX to calculate the changes in the stability of the *CYP21A2* protein. Our analysis revealed changes in the stability of the protein, in the surface charge of the mutant enzymes and effects on the active site of the enzyme that could be related to the clinical manifestation found in the patients.

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E-P16.02
F508del editing in patient-derived iPSCs by CRISPR/Cas9

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Introduction: Cystic fibrosis (CF) is a rare hereditary disease caused by mutations in *CFTR*. Most somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs). Genome editing in iPSCs provides wide opportunities for modeling and treating CF. The aim of this work was editing F508del mutation in iPSCs using CRISPR/Cas9.

Materials and Methods: iPSCs were obtained from skin fibroblasts from a patient with CF (F508del/F508del) using

Sendai virus vector carrying Yamanaka factors. Three nucleases (eSpCas9(1.1), SpCas9(HF4) and SaCas9) in combination with four sgRNAs were used. Two sgRNAs were designed to target F508del mutation (sgCFTR#1, sa_sgCFTR#3); the other two were in downstream region (sgCFTR#2, sgCFTR#3). In addition, we used a plasmid pGEM with an insert of CFTR fragment with F508del to study potential influence of the genomic context on the editing efficiency. Four single-stranded oligodeoxynucleotides (ssODNs) were designed to repair double-strand DNA breaks. Cas9+sgRNA plasmids were co-transfected with model plasmid and ssODN into iPSCs by lipofection. The editing efficacy was evaluated by TIDE and TIDER methods.

Results and conclusions: Indel formation efficacy varied from 1.4 to 9.6%. The average cut rate in pGEM-CFTR was 9.3%, while in genome locus—only 4.3%. The best combination was eSpCas9(1.1)/sgCFTR#3. CTT knock-in efficacy using different ssODNs was low (0–0.5%). Additional studies are necessary to confirm these results and increase efficacy. However even this efficiency allows to select and cultivate corrected patient-derived iPSCs to further use the for cell therapy of CF.

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E-P16.03
PanelMaps: a genome-scale coverage QC and CNV advisor web application

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Introduction: one of the main critical points in clinical grade genomics is the sequence coverage of the region of study and the ability to detect Copy Number Variants (CNV)The aim of this work is the design and development of a web tool for the visualization of coverage QC at genome and gene panel scale and detect putative region for CNV.

Materials and methods: PanelMaps is a user friendly web tool developed using modern web technologies and one of the most scalable backends for genome data management, provided by OpenCB. This web tool provides an interactive explorer for sequencing panel design and experimental coverage QC. The web tool also provides a CNV advisor for WES, WGS and targeted sequencing panels.

Results: PanelMaps is a useful tool for real time detection and visualization regions of genes altered in panels that improves the knowledge of the genetic basis of diseases and produces useful information for diagnosis in clinical contexts.

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E-P16.04
Whole exome analysis for Rheumatoid arthritis patients in three Pakistani familial effected samples

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Introduction: Rheumatoid arthritis is a multifactorial chronic autoimmune disease. To identify potential genetic casual variants, whole exome sequencing was performed in three Pakistani familial RA patients.

Materials and Methods: BWA-MEM and PICARD were used for alignment and duplicates removal respectively. GATK software was used for adjusting base quality scores in reads and then selecting mixed variants (SNP and Indel), once variants were called via de-novo assembly of

haplotypes in an active region. SnpEff and SnpSift software were used to annotate selected variants and additional "clinvar" and "dbnsfp" datasets. Finally Ingenuity Variant Analysis tool (Qiagen) was used for finding pathogenic variants and related pathways.

Results: Total SNPs, INDELS and Transitions/Transversions ratio (Ts/Tv) present in RA1, RA2 and RA3 include 95,818, 93,971 and 475,422; 13,312, 12,857 and 52,216; 2.3 and 2.2 and 1.6 respectively. From 473 combined variants in RA1 and RA2, *ALG13, MCF2L, USP17L2*, *ZNF717* genes were identified with *MCF2L* directly leading towards inflammation while other genes act through intermediates. Similarly from 290 variants in RA3, *ABR, ADRMI, APBB3, SLC35A4, CCDC7, CCDC15, CHD1, DOK3, DPY19L4, GALC, GYPC, MAML2, POLQ, SLC12A4, TMEM67, YWHAZ, ZNF331, ZNF384* genes were identified with *ZNF331* have direct effect towards RA while others act through intermediates leading towards RA and inflammation

Conclusions: Important variants in three RA familial samples are identified in genes which may cause Rheumatoid Arthritis. In future we plan to exome sequence large number of cases and controls to construct polygenic risk scores for RA.

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E-P16.05
Combining cell-specific barcoding libraries with targeted gene expression for single-cell genetic analysis

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Labeling of target cells with lentiviral barcode libraries offers an effective approach for monitoring cell phenotype in time-course experiments *in vitro* and *in vivo* using single-cell molecular analysis. Cellecta's CloneTracker libraries, with millions of different barcodes, enable rapid labeling of each cell in a large population with a unique sequence that is detectable in NGS RNA expression profiling assays such as RNA-Seq. These barcodes then provide a key to identify the subpopulation of progeny cells, and track phenotypic changes such as activation, differentiation, etc., derived from a single barcoded progenitor cell. Further, cell barcodes can be incorporated in conjunction with genetic effector libraries, such as CRISPR sgRNA libraries, to identify phenotypic changes induced in subpopulations by specific genetic disruptions in progeny cells derived from the single progenitor cell. We will demonstrate how DriverMap Targeted RNA Expression Profiling with CloneTracker lentiviral barcoded libraries, provide a complete platform for highly sensitive single-cell expression profiling. Data will be presented showing how targeted DriverMap RNA expression profiling of single cells combined with cell barcoding could significantly improve phenotyping of distinct cell populations.

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E-P16.06
Optical Genome Mapping for Detection of Structural Variants in Constitutional Disease

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Bionano Genome mapping is quickly becoming established as a key method for detection of intractable types of structural variation. Currently, genomes from many different disease states, some of which do not have a molecular diagnosis and others which are very difficult to diagnose are being studied. For example FSHD1, is caused by a repeat collapse of a tandem repeat array with unit sizes of 3.4 kilobases each. This repeat array is generally only be measured by southern blot, a labor intensive and low resolution approach. Another disease type that is difficult to diagnose is triplet expansion diseases such as Fragile X syndrome and Myotonic Dystrophy, these repeat arrays can expand to many kilobases. Microdeletions and microduplications, which cause diseases such as DiGeorge syndrome and other disease syndromes, are detectible by microarrays as well as WGS but they are caused by rearrangements between large segmental duplications that flank the region in question and the segmental duplications are extremely hard to study with conventional tools. Genome mapping can accurately assemble and assay relevant regions for each of these disease classes, even those involving very large segmental duplications. Bionano has built bioinformatics tools to effectively prioritize the ~6000 structural variants based on the estimated frequency in a control population, whether it's inherited or de novo, whether it's somatic and also its proximity to a gene. We provide several examples of pathogenic variants found through Bionano genome mapping.

A. Hastie: A. Employment (full or part-time); Significant; Bionano Genomics. **A.W.C. Pang:** A. Employment (full or

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E-P16.07

Phenomics is still more powerful than whole-exome sequencing: their role in clinical genetics

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Introduction: Whole-exome-sequencing (WES) is useful method for identifying the molecular basis of genetically heterogeneous conditions but an advanced clinical interpretation is still in the need due to large numbers of non-conclusive variants detected or limited genotype-phenotype correlations. We herein describe three cases to argue the importance of phenomics and genomics approach.

Cases: The first case was a 2 years-old hypotonic female with developmental delay and minor dimorphic features who has a gastrostomy. The cranial MRI revealed cerebellar atrophy. Her parents were consanguineous and no affected sibling. WES analysis identified a frameshift homozygous mutation in *ACTA1* gene (p.Leu112Profs*16) which was the cause of scapulohumeroperoneal myopathy, nemaline myopathy type-3 or congenital fiber-type disproportion myopathy.

The second case was a 16 years old female with progressive ataxia. The parents were consanguineous and no affected sibling. A homozygous missense mutation of *PLA2G6* gene was detected (p.Asn214Ser) which was related to infantile neuroaxonal dystrophy-1 or neurodegeneration with brain iron accumulation-2B.

The third case was a 3 years old hypotonic male with motor delay, intellectual disability and overgrowth. His parents were consanguineous and no affected sibling. A homozygous stop-gain mutation of *PIEZO2* gene (p.Arg1611*) was detected which was involved in the etiology of distal arthrogryposis type-3/5 or with impaired proprioception and touch or Marden-Walker syndrome.

Conclusion: These cases emphasizes that even when sequencing the patient's entire exomes, combined deep clinical phenotyping with WES is a must because of the human data is in many ways more complex than that for in-silica models or computational analysis.

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The first private all-Russian genome initiative - #500genomes from Zenome

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Introduction: In connection with the reduction in the price of genome sequencing, companies producing any consumer goods will be able to personalize their products and thereby increase the market size. It is not a secret that some people like more bitter foods, while others like sweeter ones. Zenome has proposed a pilot project #500genomes for the development of its own network.

Materials and Methods: In various social networks in February 2019, users were asked to leave their e-mail, age, gender and place of permanent residence if they are ready to answer questions about their health status or taste preferences in the future. On the other hand, if included in the program, they were guaranteed a genome-wide genetic test and the ability to use the Zenome.io network to interpret the results. After the collection of questionnaires was completed, commercial partners were asked to select and finance the sequencing of the test group for their projects. MGISEQ2000 was chosen as the main sequencing platform. To optimize costs, genotyping will be conducted using low pass sequencing technology and imputation methods.

Results: Until February 14, 2019, in 7 days 4200 questionnaires. 500 people were selected by various partners, who formed the sample of interest. Most of these people are healthy residents of large cities in Russia aged 18–30 years.

Conclusions: This approach allows reducing the cost of personalization of any goods by sharing the same sample when conducting a genome-wide associative study. Thus, this method becomes more accessible for companies.

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E-P17

Epigenetics - Gene regulation

E-P17.01

Hypomethylation of TNF-alpha gene promoter in patients with Cystic Fibrosis

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Objective: The aim of the present study was to investigate the epigenetic alteration in the promoter of TNF-alpha in patients with Cystic Fibrosis (CF).

Methods: Genomic DNA from activated CD4+ T-cells of 14 CF patients and 14 controls were purified and subjected to bisulfite modification using EpiTect Fast Bisulfite Kit (Qiagen, USA). Methylation status of the TNF-alpha gene was determined by methylation-specific polymerase chain reaction (MSP) analysis. PCR products were electrophoresed on 3% agarose gel and visualized. The level of serum TNF-alpha was determined by enzyme-linked immunosorbent assay (ELISA).

Results: The serum level of TNF-alpha in patients with CF was higher than that in healthy controls. In addition individuals with CF have a significantly higher percentage of hypomethylation of TNF-alfa gene promoter regions in CD4+ T-cells compared to healthy controls.

Conclusion: Our results suggest that the methylation pattern of TNF-alpha promoter could help to predict the complication of Cystic Fibrosis.

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E-P17.02
Methylation status of the Alu repetitive sequence in subclinical hypothyroidism patients with T677C variant of *MTHFR* gene

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Introduction: Epigenetic mechanisms such as DNA methylation may be related to the thyroid health. This study aimed to determine the methylation level of the Alu interspersed repetitive sequence in subclinical hypothyroidism (SH) patients with T677C variant of *MTHFR* gene.

Materials and methods: The study was approved by the ethic committee of Tbilisi State Medical University. PBMC was obtained from peripheral blood of SH patients and healthy controls by a gradient centrifugation. The *MTHFR* C677T polymorphism was genotyped by PCR- RELF method. Methylation levels of Alu in PBMC from patients and controls were examined by the combined bisulfite restriction analysis-interspersed repetitive sequences (COBRA-IRS). The bisulfite-treated DNA was subjected to 40 cycles of PCR with two primers, Alu-F (5′-GGCGCGGTGGTTTACGTTTGTAA-3′) and Alu-R (5 '-T TAATA AAAACGAAATTTCACCATATT AACCAAAC-3′).

Results: The frequency of unmethylated Cytosine $^{u}C^{u}C$ in PBMC was significantly higher in SH patients with C677T polymorphism of *MTHFR* gene compared with healthy controls indicating a hypomethylated status of Alu sequences.

Conclusions: Our results indicate that *MTHFR* 677TT genotype correlates with hypomethylation of Alu repetitive sequence in SH patients. These epigenetic changes may lead to a dysfunction of the thyroid gland and thus development of subclinical hypothyroidism.

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E-P18
Genetic epidemiology - Population genetics - Statistical methodology - Evolutionary genetics

E-P18.01
The association of *AMY1* Copy Number Variation and obesity in Korean population

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Copy number variation of the salivary amylase gene (AMY1) is known as associated with body mass index (BMI) and obesity risk. We had conducted a study in association of *AMY1* CNV with obesity indices and fat storage amount in 2,270 Koreans, known to be a homogenous and high starch diet population. We used CT-measured central adiposity as an obesity index, which can test more accurate association between *AMY1* CNV and fat storage amount. In Korean population, BMI showed no significant association with *AMY1* copy number. But, all the obesity parameters showed increasing trend of *AMY1* copy number. Obese group defined by central adiposity showed significantly higher *AMY1* copy number than that of the non-obese group. High *AMY1* copy number group (≥8 copy) showed increased levels of all obesity parameters.

Conclusively, in Korean population, *AMY1* copy number had weak but positive association with fat storage amount, contrary to the previous reports. These findings may have resulted from different level of dependence on starch or different genetic architecture among populations.

H. Son: None.

E-P18.02
Analysis of BRCA1 and BRCA2 genes mutations in women with breast cancer in Georgian population

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Introduction: The morbidity and mortality rate caused by malignant tumors stands on the second place in Georgia. Breast cancer is the most common cancer in women. BRCA1/2 gene mutations result in a hereditary cancer predisposition syndrome—elevated risk of breast and ovarian cancer. Considering the facts that there are no previous studies of BRCA1/2 mutations in Georgian population and BRCA1 gene 5382insC and 185delAG mutations and BRCA2 gene 6174delT mutation are in high frequency in different populations (most frequent in Ashkenazi Jewish), it was reasonable to investigate distribution of these specific mutations in Georgian population at the beginning of our study.

Materials and Methods: 100 Georgian women, from different regions of Georgia, under the age of 40 with the breast cancer and at least one first or second degree relatives who were suffering from breast or ovarian cancers, were genotyped by PCR analyses during five years.

Results: Existence of any above mentioned mutations was not detected among studied Georgian women. These results differ from the data of Ashkenazi Jewish and different European populations, where these mutations and especially BRCA1 gene 5382insC mutation are more distributed.

Conclusion: Due to the fact that the most prevalent deleterious mutations of different populations were not seen in Georgian women, it's reasonable to sequence whole BRCA1/2 genes for detection the major mutations which are responsible for inherited breast and ovarian cancer in Georgian population. This would give us the opportunity to draw out the recommendations for the state screening programs.

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E-P18.04
Age and sunlight exposure patterns as predictors of DNA damage in a population of Malay women

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DNA damage has been linked to human diseases. This study aimed at identifying predictors of DNA damage among apparently healthy Malay women. A cross sectional study was conducted in Malaysia. 25-hydroxyvitamin D (25 (OH)D) (VitD), lipid profiles, fasting glucose, body mass index (BMI), sleep quality, stress, depression, anxiety level, sunlight exposure patterns and protection habits, and physical activities were measured following standard protocols. The levels of DNA damage in blood lymphocytes were evaluated using single cell gel electrophoresis assay by measuring head intensity, tail intensity, tail moment, and DNA in tail. A total of 134 women were recruited (mean age 29.4 ± 6.4 years). High BMI (56%), VitD deficiency (96.3%), poor sleep quality (44%), poor sun protection habits (91%), exposure to secondhand smoke (63%), allergy (21%), central obesity (23.1%), hypertension/prehypertension (29%), high cholesterol (53%), high LDL (81%), inactivity or extremely inactivity (63.4%), mild to extreme depression (27%), and anxiety (53%) were prevalent in this cohort. There was no association between sunlight exposure duration, area and protection habit and VitD level. DNA damage was significantly higher in age group 32–35 years old compared to 20–23 ($p = 0.001$). A significant association was observed between longer sunlight exposure and DNA damage ($p = 0.032$). In conclusion, age and sun exposure patterns were predictors of DNA damage among our study participants. Higher metabolic risk factors were observed among younger age group. Further studies focusing on genome health intervention is needed. The project is funded by UCSI University (Proj-In-FMHS-029).

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E-P18.06
Allele frequency analysis of Portuguese individuals based on whole exome sequencing

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Genomic studies on single populations have been performed in a growing number of countries and regions. Most of them were initiated to enlarge clinical knowledge concerning genetic variants. Each project has reported novel variants, even for populations already included in large-scale endeavors.

Those initiatives provide a reference for genomic studies. To our knowledge, Portuguese individuals are not included in either of the two most complete populational projects, the 1000 Genomes Project (1kG) and gnomAD. We believe that a Portuguese collection of genomic information would greatly benefit genetic diagnosis of rare and congenital diseases in Portuguese patients.

We sequenced seventy exomes of Portuguese individuals with the Ion Proton technology. Reads were mapped using TMAP against the hg19 reference sequence and the variants called by the Torrent Variant Caller. Variants were inserted in a MongoDB No-SQL database and compared to the 1kG and gnomAD variant information, also uploaded to the same database.

The exomes of the Portuguese individuals contained 275,159 variants, 244,615 (88.9%) of which were found in Hardy-Weinberg Equilibrium. Among those, 47,050 (19.2%) variants were not found either on 1kG or gnomAD. Furthermore, comparative allele frequency analysis using 1kG and gnomAD populations denoted genetic differences concordant with previously described evolutionary models and historical events, validating our data.

The present study stands as a useful auxiliary reference for genetic analyses of Portuguese patients. Attaining to previous initiatives, a larger project may improve general knowledge regarding the Portuguese population variant profile.

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E-P18.07
Ethnically specific pathogenic variants in Czech Roma hereditary spastic paraplegia patients

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Introduction: In endogamous populations there may occur specific disease causing variants, which have higher frequency in comparison to other populations. Knowledge of such variants for particular diseases in specific populations is very useful for effective genetic testing. We tried to find specific pathogenic variants causing hereditary spastic paraplegia (HSP) among endogamous Czech Roma population to simplify the genetic testing of Roma patients with HSP.

Materials and methods: Targeted NGS focused on HSP genes was used for Czech Roma patients with suspected HSP testing. The presence of detected pathogenic variants was subsequently tested in the ethnically matched control group (130 anonymised DNA samples from Czech Roma controls without HSP) using real-time PCR allelic discrimination TaqMan®Assay and MLPA.

Results: We diagnosed two Czech Roma HSP patients (SPG11 and very rare SPG77) both are compound heterozygotes for two pathogenic variants. We detected four pathogenic variants in heterozygous status: c.1603-1G>A and del ex.16–18 in the *SPG11* gene and c.1082C>T (p.Pro361Leu) and del ex.1–2 in the *FARS2* gene, three of them are novel. Novel del ex.16–18 in *SPG11* gene was found also in one control sample, which presents 0.77% (1/130).

Conclusions: The presence of the novel pathogenic variant del ex.16–18 in *SPG11* gene in non-HSP Roma control proband supports the fact, that this variant could be an ethnically specific variant with higher frequency in Roma population and should be preferentially tested in Roma patients with suspected HSP especially in the sporadic cases or patients with autosomal recessive HSPs.

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E-P18.08
Four STRs of the *CFTR* gene in six Northern-Caucasus populations

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Molecular genetic analysis of four intragenic polymorphisms in *CFTR* gene IVS1CA-IVS6aGATT-IVS8CA-IVS17bCA was performed in six Northern-Caucasus populations from Russian Federation. 113 Ossetians, 100 Chechens, 51 Karachai, 60 Nogai, 60 Cherkess, 57 Abaza were surveyed. Allele and genotype frequencies, indices of intrapopulational differentiation (Fst), genetic distances were calculated by Popgen32 software. The intrapopulational differentiation for all studied populations was 0.0596. The intrapopulation differentiation of the six ethnic groups of the North Caucasus by the studied markers was largely due to the significant genetic distance between the Chechen population and all the studied populations (Fst 0.0615-0.0942). Analysis of the dendrograms based on the genetic distances matrices revealed that the most genetically similar populations were the Abaza and the Cherkess (Fst = 0.0075), which is consistent with their ethnogenesis. The sequential joining of the populations of the Karachais, Nogais and Ossetians to the cluster of Abaza and Cherkess formed a common cluster to which the last stage was joined by the population of Chechens. The greatest intrapopulation differentiation of the six ethnic groups of the North Caucasus by the studied markers is due to the genetic remoteness of the Chechen population from all the studied populations (Fst 0.615–0.0942). So we have confirmed the genetic delineation of the Chechen population from other ethnic groups of the Northern Caucasus. The research was supported by grant RSF № 17-15-01051 and within the state task of the Ministry of education and science of Russia.

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E-P18.09
Distinct genetic diversity and heterogeneity of the Iranian population

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Iran, despite its size, geographic location and past cultural influence, has largely been a blind spot for human population genetic studies. With only sparse genetic information on the Iranian population available, we pursued its genome-wide and geographic characterization based on 1021 samples from eleven ethnic groups. We show that Iranians, while close to neighboring populations, present distinct genetic variation consistent with long-standing genetic continuity, harbor high heterogeneity and different levels of consanguinity, fall apart into clusters of similar groups and of admixed ones and have experienced numerous language adoption events in the past. Our findings render Iran an important source for human genetic variation in Western and Central Asia, will guide adequate study sampling and assist the interpretation of putative

disease-implicated genetic variation. Given Iran's internal genetic heterogeneity, future studies will have to consider ethnic affiliations and possible admixture.

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E-P18.10

A deep characterization of the *LILRB2/ILT4* genetic variability in Brazilian samples with different ancestry compositions

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Introduction: *LILRB2* (*ILT4*) gene encodes an NK cell inhibitory receptor targeted by HLA-G, an immunomodulatory molecule expressed in the trophoblast. Polymorphisms at both ligand and receptor might influence their binding. Although *HLA-G* variability has been explored, *ILT4* variability is unknown. We evaluated *ILT4* variability at exons in 431 samples from Brazil, using Haloplex and Illumina sequencing.

Methods: To minimize mapping bias typical of LIR genes, we developed a program to score reads based on known sequences and to address them to the correct locus. We assessed ancestry using the SNP*for*ID 34-plex panel. Samples were stratified into three subgroups: EUR ($n = 267$), AFR ($n = 51$), and EAS ($n = 13$), with major European, African, and Asian ancestries (>50%), respectively.

Results: We detected 41 variants arranged into 58 coding sequences and encoding 38 proteins. We found 24 non-synonymous mutations, 12 encompassing a polymorphic segment between positions 290 to 350 (D3/D4 domains). Many variants within this segment presented different frequencies among groups (>25%). For instance, rs7247025 is related to three amino acids at position 349, with allele G encoding Arg, A encoding Trp, and C encoding Gly. The frequencies for Arg, Trp, and Gly were 63.7, 5, and 31.3% for AFR, but 93.3, 0.94, and 5.8% for EUR. EAS presented only Arg.

Conclusion: ILT4 protein frequencies are strongly influenced by the sample ancestry background. None of the ILT4 sites involved with the HLA-G interaction was polymorphic. Further studies are necessary to elucidate if the frequency shifts result from selective pressures or genetic drift.

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E-P18.11

Next generation sequencing and genotyping in a family-based and population-based study identifying associations with Lipoedema

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Lipoedema is a chronic condition, which is characterised by an unusual amount of subcutaneous fat depositing underneath the skin particular of the hips. Little is known about lipoedema or how prevalent it is. It seems to just affect women, who report painful legs and discomfort. It is often misdiagnosed as obesity, but dieting does not reduce the size of the legs. Efforts to uncover a common genetic cause of lipoedema have so far failed. To investigate genetic variants associated with lipoedema, we recruited families

and sporadic cases at St. George's Hospital. The lipoedema patients were selected using the following main criteria: BMI of less than 35 (kg/m2) and a waist:hip ratio (WHR) <0.80. We recruited 247 cases of which 212 recruits of white British descent were genotyped using the Human CoreExome 24v.1.1 array (Illumina). Whole genome sequencing (WGS) was carried out in 91 recruits through the Genomics England '100,000 Genomes Project' and other 83 underwent whole exome sequencing (WES). First, we analysed the WGS and WES data and our results indicate there is a high locus heterogeneity. We have identified a couple of variants in our large families and functional studies are being carried out. The genotype data obtained will be used for association analysis using PLINK for the identification of further loci. Currently, liposuction is the only relief from symptoms. Understanding the underlying causes for lipoedema could help the development of treatments, thus improving the quality of life for patients. *This study is supported by the Lipedema Foundation*.

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E-P18.12
Variants associated with longevity: a case-control study in Bulgarians

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Introduction: In human longevity studies, single nucleotide polymorphism (SNP) analyses have identified a large number of genetic variants with small effects, but these are not easily replicable in different populations.

Materials & Methods: We constructed two DNA pools, one with 32 Bulgarian centenarians and the other with 61 young and healthy Bulgarian individuals. Whole-exome sequencing was performed and allele frequencies were estimated for 59935 variants detected in the two pools after robust quality filtering. Fisher's exact test was used for estimating the significance in the difference for each variant between the two pools. The publicly available database LongevityMap was used to set up a list of variants associated with longevity, and we screened our data for the presence of such variants.

Results: Forty two out of the 555 longevity associated variants (LAV) listed in the LongevityMap database were discovered in our pool data. Nine of these showed significant difference in allele frequencies between the centenarians and controls (p-values and FDR adj. p-values < 0.05). We choose variants to be LAV if their population frequency is < 0.5. Two variants are positive LAV: rs507879 in *CASP5* with frequency of variant allele in centenarians/controls 0.484/0.371 and rs2072454 in *EGFR*—0553/0.438. Three are negative LAV: rs660339 in *UCP2* (0.314/0.460), rs2498804 in *SIVA1_AKT1* (0.264/0.392) and rs1042719 in *ADRB2* (0.298/0.377).

Conclusions: From the 42 LAV discovered in Bulgarian case/control study only two were evaluated as positive LAV and three as negative LAV. Acknowledgment to DN 03/7 from 18.12.2016—National Science Fund

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E-P18.13
Genetic Structure and Long-range Familial Searching analysis in local Central Asian population

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Introduction: In current research, high-precision analyses of population structure and ancestral inference need large-scale population sampling and dense genomic SNP typing. In order to obtain an unrelated individual sample set, pairwise kinship analysis and close relative elimination is ought to be performed for a large-scale local population sample.

Methods: We recruited 713 Central Asian individuals from a local area of Xinjiang province. All the samples were genotyped by an Illumina genotype array that probes around ~700,000 genomic SNPs. Population structure was accessed by using ADMIXTURE. About 270,000 SNPs was for kinship analysis, and 1st cousins were eliminated from the population. Information record and STR typing were used for comparison analysis.

Results: The population of the 713 Central Asian individuals demonstrates typical European (EUR)-East Asian (EAS) admixed structure, EUR:EAS = 41.1:48.6. SNP kinship analysis shows 1C to 3C degree relatives. By searching the information records provided by the participants, there are 570 pairs of different relatives ranged from 1C to 2C degree. Compared with the records, the deviation

of the SNP-based pairwise kinship analysis is 0.512 ± 0.547 (mean ± standard deviation, ranged from 0 to 2).

Conclusion: SNP-based pairwise kinship analysis will serve as an effective tool in population genetic researches and forensic long-range familial searches.

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E-P18.14
Association of microRNA Polymoprhisms (miR-146a, miR-149, miR-196a2 and miR-499) with risk of Coronary Artery Disease

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Background: Several microRNA polymorphisms have been associated with susceptibility to many diseases, including cardiovascular diseases. The aim of the present study was to investigate whether the miRNA polymorphisms miR-146a rs2910164G>C, miR-149 rs2292832C>T, miR-196a2 rs11614913C>T and miR-499 rs3746444A>G contribute to the risk for the development of Coronary Artery Disease (CAD) in the Greek population and assess their clinical applications for diagnosing and monitoring CAD.

Material and methods: We used a case-control study to examine these associations in 200 CAD patients and 200 healthy control individuals, all of Greek origin. MiRNA polymorphisms were genotyped using PCR-RLFP, HRM (High resolution Melt) and Sanger Sequencing methods.

Results: Two of these polymorphisms, miR-196a2 rs11614913C>T and miR-499 rs3746444A>G were found to be strongly associated with increased risk for CAD ($P = 0.0388$ and $P = 0.0013$, respectively). Furthermore, miR146C-miR149C-miR196T-miR499G allele combination was also found to be significantly associated with CAD prevalence ($p = 0.0185$).

Conclusions: Our results suggested that the studied miR-196a2 and miR-499 polymorphisms may represent useful biomarkers of CAD susceptibility in the Greek population and with combined effects of environmental factors might contribute to CAD prevalence.

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E-P18.16
Polymorphism of nine nuclear genome DNA loci in Ossetian population

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The Ossetians are a unique ethnic group of the Caucasus, living both on the north and south slopes of the Caucasus mountain range. They speak Ossetic, an Eastern Iranian (Alanic) language of the Indo-European languages family. DNA of 100 healthy unrelated North Ossetians (78—from Vladikavkaz, 22—from other regions of the Republic of North Ossetia—Alania) were analyzed for 9 autosomal DNA markers (diallelic and multiallelic): *CCR5(del32)*, *ACE(del/ins)*, *D7S23(KM19)*, *THOI(STR)*, *FABP2(STR)*, *CFTR(STR)*, *PAH(VNTR)*, *DAT (VNTR)*, *NOS3(VNTR)*. An estimation of frequency distribution of alleles and genotypes of the studied polymorphic loci was obtained. The highest level of genetic diversity for diallelic systems was shown for locus *ACE(del/ins)*, $H_{obs} = 0.4358$, for multiallelic system—for locus *THOI(STR)*, $H_{obs} = 0.8400$. The index of mean heterozygosity is 0.4490. Based on the data of allele frequencies of the nine studied polymorphic DNA loci, a matrix of genetic distances between five North Caucasus and six Volga-Ural populations was obtained. The analysis of dendrograms, based on correlations between the matrix of genetic distances, and multidimentional scaling analysis was carried out. The population of Ossetians forms a single cluster with the population of Cherkess, then joins the cluster of Abaza and Nogai populations, the population of Karachays turned out to be more genetically distant. The populations of the North Caucasus, as well as the populations of the Volga-Ural region, form separate clusters. The research was supported by grant RSF grant №17-15-01051 and within the state task of the Ministry of education and science of Russia.

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E-P18.18

Analysis of carriership of alleles in tumor suppressor genes in healthy Bulgarian individuals

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Introduction: Tumor suppressor genes (TS) predispose to inherited forms of many cancers. The aim of our study was to evaluate the presence and frequencies of variants in 181 TS genes in healthy Bulgarian subjects of different age.

Materials and methods: DNA samples from 93 healthy Bulgarian subjects (32 centenarians, aged ≥ 100 years and from 61 healthy controls (18–30 years)) were extracted and whole exome sequenced. Uniprot and dbSNP databases were used to select 29 000 variants in 181 TS genes. The frequency of the variants found in our samples was examined and their clinical significance was reevaluated.

Results: 13 out of 29 000 SNPs were found to be present in our dataset of healthy subjects: *BRCA2*—rs543304 (23%), rs1799955 (19%), rs2126042 (18%), rs144848 (20%); *PROC*—rs5937 (23%), rs1799810 (40%), rs5936 (63%); *NF1*—rs1801052 (71%); *SDHA*—rs2288461 (82%); *STK11*—rs58579265 (23%); *MSH2*—rs2303426 (42%); *PTCH1*—rs2066836 (18%) and *DFNA5*—rs17149912 (17%). Eleven are currently classified in public databases as "likely benign" and two (*BRCA2* rs2126042 and rs144848) are with "uncertain significance" (VUS). Pathogenic and likely pathogenic variants were not found.

Conclusion: None of our 93 healthy subjects (both centenarians and young individuals) carry pathogenic/likely pathogenic variants of the analyzed TS genes. Based on the frequency of the detected variants in our dataset (centenarians/controls), we could reclassify the "likely benign" variants to "benign" (eleven) and "VUS" to "likely benign" (two).

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E-P18.19

Development of a Y-SNP array based on major Y-haplotypes using multiplex PCR and SNaPshot minisequencing and validating on a few selected Sinhalese and Sri Lankan Tamil individuals

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Introduction: Sinhalese and Sri Lankan Tamils are two major ethnic groups living in Sri Lanka with an extended history. Extensive studies have not been carried out to trace back their geographical origin based on the paternal ancestry. Hence, the present study was mainly focused on developing a Y chromosomal single nucleotide polymorphism (Y-SNP) array to investigate Y haplotypes diversity in these two ethnicities.

Materials and Method: Sixteen Y-SNPs were selected according to the historical relationships and migration patterns of Sri Lankans. They cover major haplogroups specific to Indo-European and Dravidian language speakers and indigenous tribal populations. The Y-SNP genotyping was accomplished by co-amplifying 16 fragments in 3 multiplex PCRs followed by respective minisequencing/single base extension reactions using SNaPshot™ multiplex kit and capillary electrophoresis.

Results: Genotyping using SNaPshot™ minisequencing was successful for 13 Y-SNPs and three markers required further optimizations. According to the results of 15 samples [Sinhalese (N = 10) and Sri Lankan Tamils (N = 5)], 13 different lineages specific Y chromosomal SNPs indicate that Sinhalese population consists of Y-haplogroups F, H, R2, R1a and R1b while Sri Lankan Tamils consist of F, H, J2, R1a and R2.

Conclusions: Y haplotype diversity does not display a significant difference between Sinhalese and Sri Lankan Tamils studied. However in order to arrive at solid conclusions on the Y- haplogroup structure and paternal migration patterns of these two populations a higher number of samples need to be analyzed. This work was supported by IBMBB, University of Colombo, Sri Lanka

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E-P19

Genetic counselling - Services - Education

E-P19.01

ATP7B gene mutation frequencies among Wilson's disease patients from Ukraine

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Introduction: Wilson disease (WD) is an autosomal-recessive disorder of hepatocellular copper deposition caused by pathogenic variants *ATP7B* gene. Genetic testing of *ATP7B* gene is not usually used for Wilson's disease (WD) diagnostics in Ukraine and due to the marked heterogeneity in clinical presentation the diagnosis of WD remains challenging. The objective was to establish the frequency and range of *ATP7B* gene allelic variants among Ukrainian patients.

Materials and methods: Analysis of H1069Q was performed among 85 patients with signs of WD by PCR BI-PASA. Exons 1–21of the *ATP7B* gene were directly sequenced among 23 WD suspicion patients. HRM (high resolution melting) analysis of exons 8 and 14 was tested for screening of common allelic variants. RFLP analysis of exon 8 allelic variants (c.2304dupC, c.2128G>A) was developed.

Results: The most prevalent *ATP7B* gene allelic variant c.3207C>A (p.H1069Q) in Europe was informative for 18 patients out of 23 WD cases. 11 (47.8%) WD patients were homozygous for H1069Q mutations, 7 heterozygous with compound of other *ATP7B* allelic variant: 3—c.2304dupC (ex 8), 1—c.2128G> A (ex 8), 1—c.3011A>C (ex 13), 1—c.3402delC (ex 15), 2—unidentified. The most common H1069Q mutation covers 63.0% of mutant allele; 8.7%—c.2304dupC; 2.2%—c.2128G> A; 2.2%—c.3011A>C; 2.2%—c.3402delC.

Conclusions: 1) The most common allelic variant among Ukrainian WD patients was H1069Q; 2) HRM analysis of exons 8 and 14 was elaborated for screening.

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E-P19.02
A folder as an educational instrument: Fragile X Syndrome

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Introduction: The Fragile X Syndrome (FRAXA) is the second most common cause of genetic intellectual deficiency in boys; however, it is not widely known and in developing countries is underdiagnosed. The cause of the syndrome is an alteration in the 5′UTR region of *FMR1*'s gene resulting from an unusual repetition of CGG bases, which increase over generations. Thus, the main aim of this folder was to inform the general population, as well as the health professionals, about the main FRAXA features and risks.

Materials and Methods: It was prepared an educational folder aimed at health professionals and students containing information about the genetics etiology of this syndrome, its signs and symptoms, how its diagnosis and the genetic counseling is performed. This folder was used in a Genetics' undergraduate courses.

Results: The students demonstrated a good acquisition of the syndrome symptoms, etiology and its genetic counseling, confirming the efficacy of the folder.

Conclusion: The created folder has demonstrated to be a useful and efficient tool for the propagation of knowledge about FRAXA.

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Testing for heredity in Parkinson's disease - Opinions of patients and neurologists

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We performed a study to explore the views of patients with Parkinson's disease, and their neurologists, about genetic testing for hereditary causes of this disease. Patients were asked if they were they willing to be tested, how they were informed about the option of genetic testing and what information they needed at which moment. Neurologists were asked how they provide information about genetic testing and what information needs they have.

Materials and method: Semi-structured interviews were held between September 2017 and January 2018. Participants were patients with Parkinson's disease, diagnosed before their 50th year of life and/or with at least one first-degree relative with Parkinson's disease (n = 13). An online

survey was filled in by neurologists with experience with Parkinson's disease (n = 10).

Results: Both patients and neurologists would like to have more information tools, for example an extensive information folder. Patients would like to receive all information available about their disease from their neurologist and want to be able to find more information online. Patients also think the option of genetic testing should always be mentioned.

Conclusions: There is need of more written information to help neurologists and patients discuss the genetics of Parkinson's disease. Neurologists should mention the topic of heredity in Parkinson's disease to all patients, but preferably not in the first visit. More information can be provided at later stages. Online information about Parkinson's disease should be easier to find and read for lay people. A clear and extensive website should therefore be made.

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By origin genetic screening testing is insufficient for the complexity of human nature

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Introduction: Rare genetic diseases have been reported with high frequency in many genetically isolated populations. When all the affected patients have only one frequent mutation, a common founder effect is the most reasonable explanation. Information on the frequency and distribution of genetic diseases in the Israeli population was the basis for a national carrier screening program for the prevention of severe genetic hereditary disorders, according to the ethnicity and residence.

Results: We present a patient with mental retardation and complicated spastic paraparesis, an offspring of first degree cousins from a village in northern Israel. This village holds Druze, Christian Arabs and Muslim families. Whole Exome Sequencing yielding a nonsense homozygous SPG11 gene mutation related to hereditary spastic paraparesis type 11. Surprisingly, this mutation was familiar among other ethnic local community and was part of the carrier screening program of this ethnic group.

Conclusion: We discuss the limitation of "ethnicity based" screening programs, and recommend accelerating a national expanded carrier screening in Israel.

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