



ABSTRACT

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E-P01 Reproductive Genetics/Prenatal Genetics

E-P01.02

Prenatal diagnosis in a case of 8p inverted duplication deletion syndrome

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Introduction: 8p inverted duplication deletion syndrome is a rare chromosomal anomaly characterized by mild to severe intellectual deficit, severe developmental delay, hypotonia, agenesis of the corpus callosum and minor facial anomalies such as prominent forehead, temporal baldness, anteverted nostrils and eversion of the lower lip. Materials and Method: A prenatal case was investigated due to high risk for Down syndrome in first trimester biochemical screening and advanced maternal age (36) by amniocentesis and fetal ultrasonography (USG). Results: Amniocentesis was performed at 16th weeks of gestation. The result of the QF-PCR analysis for rapid aneuploidy screening was normal, but add (8p) was determined by conventional karyotyping. The parental karyotypes had no chromosomal anomaly. In the subtelomeric FISH study of the fetal sample, there was a subtelomeric deletion in the 8p region. There was no fetal structural anomaly in level II fetal USG

and fetal echocardiography. The molecular karyotyping revealed a gain in 8p11.22-p23.1 region with a size of 27.2 Mb containing 122 OMIM gene and a loss in 8p23.1-p23.3 region with a size of 6.8 Mb containing 15 OMIM gene. The findings were correlated with 8p inverted duplication deletion syndrome. Conclusion: Our study emphasizes the importance of using additional molecular cytogenetic methods in clinical follow-up of complex rearrangements in the prenatal cases. In unstable chromosomal rearrangements, fetal USG scans are not sufficient to detect fetal anomalies. However, molecular karyotyping is gaining importance in order to evaluate unbalanced chromosomal anomalies, to predict fetal prognosis and to give appropriate genetic counseling.

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E-P01.03

A Case of Fertile Man With Lack of AmelogeninY Locus

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Introduction: Amelogenin gene is responsible for amelogenesis and the development of enamel of tooth. The gene is located each of the sex chromosomes. AmelX is present on the Xp22.1-p22.3 and AmelY is present on the Yp11.2, differ by a 6 bp deletion in the third intron of AmelX is not in AmelY. It is lead to be useful for gender identification detecting of amelogenin homologus. It's fact that Y chromosome abnormalities are primarily considered for male infertility or subfertility. Materials and Methods: Quantitative fluorescent polymerase chain reaction (qf PCR) combination with conventional cytogenetic analysis has been used routinely in prenatal diagnosis. The technique, is based on the length variations of short tandem repeat (STR) in chromosomes. Also, AmelX and AmelY sequences are these markers. In the case, fourteen weeks chorionic villus samples (CVS) with indications of omphalocele and anencephaly were referred to our clinic for prenatal diagnosis. When detecting the lack of AmelY locus in CVS we analyzed the parents immediately for amel fragments. Results: AmelX of the mother was normal level whether fetus and father have not any size of AmelY loci but had SRY sex-determining region Y marker. Conclusion: A few cases have been reported interstitial deletion of AmelY loci have oligozoospermia clinique associated with male infertility but we had described lack of AmelY locus both fetus and the father. Interesting feature of the case that father had not dysmorphic features also is a fertile man. Genetic counseling was given to the family.

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E-P01.04

QFPCR in Invasive Prenatal Diagnosis: Single Center Experience in Turkey

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QFPCR is being used for more than 20 years. It is based on the investigation of polymorphic short tandem repeats (STRs) and is preferred widely for prenatal rapid aneuploidy detection. In this study, we reported retrospectively our prenatal diagnosis results between January 2012 and May 2014 in Tepecik Training and Research Hospital Genetic Diagnostic Center. Prenatal diagnosis was offered to 6800 high risk pregnancies and 2883 cases accepted

invasive diagnosis. Chromosome analysis and QFPCR was performed for all patients. Normal results were reported in 2711 cases by fetal karyotyping and in 2706 cases by QFPCR. Anomaly detection rates were similar for two methods (5,09% for karyotyping and 4,02% for QFPCR). QFPCR as a fast and reliable prenatal diagnosis method in all indication groups and may be preferred as the sole prenatal investigation in patients without fetal ultrasonographic findings.

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E-P01.05

Cell-free fetal DNA in amniotic fluid supernatant for prenatal diagnosis

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Amniotic fluid is widely utilized for prenatal diagnosis. Amniotic fluid supernatant however, is usually discarded and not deemed good source of fetal DNA. The aim of this study was to assess cell-free fetal DNA extracted from amniotic fluid supernatant for application in prenatal diagnosis such as gender determination and early diagnosis of β-thalassemia. Samples of amniotic fluid from 70 pregnant women were collected and put through routine tests along with tests for cell-free fetal DNA from the amniotic fluid supernatant. The DNA in the amniotic fluid supernatant was extracted and analyzed for gender determination by PCR and Real-time PCR. ARMS-PCR was applied to test for the early diagnosis of IVS II-I mutation (common β-thalassemia mutation) and E7V mutation for sickle cell anemia using DNA extracted from the amniotic fluid supernatant. Using the cell-free fetal DNA extracted from the amniotic fluid supernatant, the sensitivity of PCR and Real-time PCR for gender detection was compared with the routine cytogenetic method. The fetus tested for sickle cell anemia and β-thalassemia was observed to be healthy but heterozygous for IVS II-I mutation. The findings indicated that cell-free fetal DNA from amniotic fluid supernatant can be a good source of fetal DNA and be used in early prenatal diagnosis due to its fast and accurate application. Therefore, it would be suggested that the supernatant from the amniotic fluids' disposal is prevented because if the tests needs to be

repeated, cell-free fetal DNA extracted from the amniotic fluid supernatant can be used as an alternative source for prenatal diagnosis.

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E-P01.06

A multidisciplinary approach in a two generation family presenting with a 46,XY disorder of sex development

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Introduction: 46,XY disorders of sex development (DSD) are caused by alterations in gonadal development, androgen receptor or androgen biosynthesis. Androgen Insensitivity Syndrome (AIS) is an X-linked disorder caused by pathogenic variants in *AR* gene. The aim of this work was to describe a family presenting with DSD and all the multidisciplinary approaches that were needed to achieve the precise diagnosis and correct follow-up. Materials and Methods: Both clinical and biochemical characteristics of three individuals from two generations of a family presenting with DSD are described. Karyotype, SRY FISH and sequence analysis from *AR* or *SRD5A2* genes were performed according to the clinical clues. Results: Our index case, a 17 years old girl, presented with primary amenorrhea, normal female external genitalia, absence uterus on MRI and 46,XY karyotype. Hormonal studies revealed high delta-4-androstenedione, very high testosterone and low dihydrotestosterone. Her maternal aunt and her young sister have the same phenotype. A pathogenic variant c.1508G>A in exon 1 of *AR* gene was found in hemizygosity in all patients and in heterozygosity in the mother. A secondary 5- α -reductase deficiency could explain the hormonal profile detected in our AIS patients. The management includes hormone replacement therapy,

prophylactic gonadectomy, psychological support and genetic counselling.

Conclusions: DSD are rare however extremely significant conditions that may interfere with life quality, so all efforts should be made in order to get the precise diagnosis as well as the best care possible.

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E-P01.07

A Novel Mutation Of Androgen Receptor Gene In A Primary Amenorrhoea Patient

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Introduction: Androjen receptor (AR) gene is located Xq11-q12 and the gene encoding the androgen receptor. At last, over 1,000 mutations have been reported in AR gene and with most of these being associated with incompatable genotype and phenotypes. Clinical findings are external genitalia at birth, abnormal secondary sexual development in puberty, and infertility in individuals with a 46,XY karyotype as typically characterized androgen insensitivity syndrome. Materials and Methods: In our case, 17 year old female phenotype presented with primary amenorrhoea and the predominantly female external genitalia. Patient, had spontaneous once menarche but not continued. Radiology results had demonstrated the absence of uterus and vagina, only right ovary available. Firstly, we analyzed karyotype, then detected the gene with sequence analysis and Multiplex Ligation-dependent Probe Amplification (MLPA) to exclude large deletions and duplications. Results: Patient had 46, XY karyotype and female phenotype, MLPA test is normal but the results of the gene sequence is not. We detected a missense mutation in first ekson. GAA codon was turned into GGA. Substituting at position 529 to glutamic acid exchange to glycine. NM000044 c.5 A>G variant was neither found in ExAc or 1000 genome base. Conclusion: We have detected a novel mutation on AR gene immediately after start codon at fifth nucleotide. Glutamic acid is polar aminoacid with negative charge while glycine is stayed in non polar hydrophobic group.

Lastly, the mutation may cause to physical defect on protein and native three dimensional structure.

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Array CGH in prenatal diagnosis versus karyotype. our personal experience

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The clinical utility of array comparative genomic hybridization (CGH) in the prenatal samples has been proved to be effective, but it is still in discussion on how to apply array CGH into clinical service, and whether array CGH should replace the conventional cytogenetics. A total of 118 prenatal samples from patients with abnormal ultrasound were selected, 58 of them were chorionic villus sampling (CVS) and 60 amniocentesis. Karyotype and array CGH was performed in all of them. Karyotype was performed according with the Giemsa standard technique. Array CGH with a 180 K resolution, was performed with the Nimblegen CGX Cytogenetic Microarrays Platform, supplied by PerkinElmer. From the 118 cases, in 9 (8.1%) was detected a chromosomal abnormality, but when array CGH was performed, the number of samples with pathogenic chromosomal anomalies increased to 20 (17%), in all cases where a pathologic karyotype was detected (9 cases), the array CGH detected the same pathologic chromosomal alteration. When karyotype was normal, in 11 cases the array detected microduplications or microdeletions that can be considered pathologies (CNAs). Our results with an increase in the detection rate are in accordance with previous publications were the pathological chromosomal abnormalities detected with array CGH is highly increased when compared with karyotype. This significantly increased detection of chromosome anomalies, from 8.1 to 17%, after applying microarray analysis for prenatal testing with abnormal ultrasound supports the use of arrays as first tier test for prenatal diagnosis in cases with abnormal ultrasound detected.

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E-P01.12

Improvement of first trimester combined chromosomal aneuploidy screening by using the QUAD strategy

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Introduction: First trimester biochemical screening, using ultrasound, PAPP-A, free β-hCG (DUAL-) was supplemented by PLGF and AFP biomarkers (QUAD strategy) in order to achieve a 98% success rate for the detection of trisomy 21 (T21). We retrospectively compared QUAD vs. DUAL strategies. **Material and Methods:** Forty-seven T21 pregnancies (ascertained 2008–2016) by the DUAL (Kryptor; ThermoFischer Sci.; USA) were re-examined using QUAD (Delphia Xpress -LifeCycle software; Perkin Elmer; USA) for final risk (FR) and biochemistry only (BO) ascertainment with cut offs- 1:300 (positivity) and 1:301–1:2000 (intermediate risk). Biomarker MOM levels, age-related risk, higher NT were also considered. **Results:** QUAD FR for 1:300 was detected in 72.34% of all cases, BO in 87.23%, while in DUAL 59.57% and 65.95%, respectively. Integration of FR with BO in QUAD increased positivity to 91.49%, while in DUAL to 74.46%. If 3 cases with QUAD FR 1:337, BO 1:390, age risk 1:77, higher NT and age risk 1:458 had been included, QUAD might achieve 97.87% T21 detection rate. However, if 5 cases with DUAL BO within 1:459–1:810 had been taken into account, this rate would be only 85.10%. QUAD FR in >1:2000 was 6.38%, while in DUAL it was 19.15%, with BO 6.38%. DUAL achieved lower 21.28% PAPP-A prevalence versus 55.32% in QUAD. Only in one case all makers were normal in QUAD / DUAL.

Conclusions: QUAD provides significantly higher detection rate and reliability within 1:300 risk and achieves better screening outcomes than DUAL. Supported by 00064203, CZ.2.16/3.1.00/24022, NF-CZ11-PDP-3-003-2014.

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E-P01.13**BRCA mutations and pregnancy. Case report**

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Background. Women who inherit BRCA1 or BRCA2 mutations have a 50%–80% lifetime risk of developing breast cancer and a 16%–65% lifetime risk for ovarian cancer. Many women need genetic counseling and testing to learn about their risk status and its implications. Although most women who seek counseling and testing are past reproductive age, some still are in their childbearing years.

Case report. A 32 year old female at 23 weeks gestational age (G.A) with breast cancer diagnosis presented for genetic testing and counseling. Histopathological examination indicated an invasive ductal carcinoma with triple-negative cancer (cancer stage 2B [pT3N0M0]). The patient's mother died at age 47 due to breast cancer and denied any use of alcohol or cigarettes. She had her regular menstrual periods and never used oral contraceptives. Her menarche was at 11 years of age. Her family history, young-onset disease, and histopathological findings suggested HBOC (Hereditary Predisposition to Breast and Ovarian Cancer syndrome). She underwent genetic testing for BRCA1/2, though genetic counseling was provided. The result was positive for a BRCA2 mutation. The patient wishes to know if pregnancy impacts upon their future risks of cancer recurrence and survival. **Conclusion.** In cases of pregnancy-related breast cancer, consideration must be given to whether the pregnancy should be continued and to posttreatment fertility. Genetic counseling should be provided and the patient should be checked for the BRCA mutation, as it is meaningful for the future of any potential children and should be provided even if the cancer is advanced or recurrent.

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E-P01.14**A successful pregnancy from a tetragametic chimeric infertile man**

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Introduction: We report a case of infertile male tetragametic chimera with true hermaphroditism. Materials and methods: Karyotyping was routinely performed by G-banding using the Trypsin-Giemsa staining technique. Fluorescence in situ hybridization was performed on sperm and buccal cells. Results: A couple had 3-year history of infertility. The husband had criptozospermia. The right testis volume w as 6,8 sm³, the left - 6,0 sm³. Karyotype of lymphocytes: 46,XX [34]/46,XY[66]. Cells of buccal epithelium were mosaic too. Molecular-genetic analysis of short tandem repeats was made and tetragametic chimera was diagnosed (table). Table

MRI examination showed bilateral ovarian similar mass near the urinary bladder and prostate gland. True hermaphrodite was detected. IVF ICSI was performed using chimeras' sperm. Successful pregnancy was diagnosed and terminated term by cesarean section. **1 O. Prybushenia:** None. **E. Golovataya:** None. **S. Kotova:** None.

autosomal marks	father	mother	proband	sibs male
TPOX	8/11	8/11	8/11	8/11
D2S1338	17/25	17/19	17/19	17/19
D3S1358	17/18	15/17	15/17/18	15/17
FGA	20/20	20/23	20/23	20/23
CSF1PO	12/13	12/13	12/13	12/13
D5S818	9/12	11/11	9/11	11/12
SE33	29.2/30.212/17	12/17/29.2/30.217/29.2		
D7S820	11/11	8/12	8/11/12	8/11
D8S1179	11/13	10/10	10/13	10/13
D10S1248	14/16	15/15	14/15/16	—
TH01	9/9.3	7/9	7/9	7/9.3
vWA	17/17	16/17	16/17	17/17
D13S317	8/12	11/11	8/11	11/12
D14S1434	10/13	13/13	10/13	—
Penta E	12/14	13/18	12/13/14/18	14/18
D16S539	9/11	9/11	9/11	9/11
D18S51	15/17	15/16	16/17	16/17
D19S433	13/13	16/16.2	13/16.2	13/16
D21S11	28/30	29/29	29/30	29/30
D22S1045	16/16	11/17	11/16/17	—
F13B	168/184	180/184	180/184	180/184
Penta B	137/142	118/118	118/137	118/142
Penta C	133/133	123/123	123/133	123/133
Amelogenin X	XY	XX	XY	XY

E-P01.15**Types and frequency of chromosome aberrations in Bulgarian patients with infertility**

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Background: Reproductive genetics combines reproductive and genetic technologies aiming in precise diagnostics and the most suitable therapeutic options, as well as prevention of birth of children with congenital anomalies and genetic diseases. We aimed at determining the types and frequency of the chromosome aberrations in patients with reproductive failure - sterility, miscarriages, stillbirths, and implantation failures after in vitro procedures. Materials and Methods: We have analysed 488 patients from our reproductive clinics by karyotype analysis using G-banding technique. Results: We detected 21 (4.3%) cytogenetic aberrations, both numerical and structural. Klinefelter syndrome was revealed in 2 out of 27 azoospermic men (7.4%). Structural chromosomal rearrangements were established in 4 out of 258 women (1.6%), and in 3 out of 230 men (1.3%). One complex three-way translocation was detected in a woman, it has familial character - 46, XX, t(1p31- > 11q22- > 8q12- > 1p31). The other translocations included: 46, XX, t(7; 10)(q11; p13); 46, XX, t(9; 11)(p23; p12); 46, XX, t(11; 22)(q23; q11.2); 46, XY, t(5, 6)(p13; q16); 46, XY, t(1; 11)(p36.1; q12). One ring chromosome 21 (46, XY, r(21)) was found in an azoospermic man. In 1% of tested patients we found inversion of chromosome 9. Sex chromosome low level mosaicism was found in 1% as well. Conclusions: In about 5% of patients attending reproductive clinics cytogenetic abnormalities were revealed. They have important impact for therapy decision making. In two of translocation carriers successful pregnancy was realized by means of preimplantation genetic diagnostics.

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E-P01.16**Chromothripsis and hypodiploidy in abortive materials from malformed fetuses**

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Introduction: Chromothripsis is a mutational mechanism in which powerful rupture of chromosomes in different points occurs in a cell cycle, leading to chromosome fragmentation and subsequent uneven connections. As a result, many parts of chromosomes remain deleted. The rupture is due to external or internal mutagenic factors such as ionizing radiation, free radicals, and mutations in the genes for cell repair mechanisms. The consequences are associated with impaired apoptosis, cell division and survival. Impaired apoptosis in turn leads to hypodiploidy. Materials and methods: We report here 4 cases with ultrasound or cytogenetic anomalies of the fetus, which have been analyzed by array CGH analysis. Results: In a case with multiple development anomalies (agenesis of corpus callosum, tetralogy of Fallot, omphalocele) we detected multiple genomic deletions, varied from del1p36 to monosomies of chromosomes 15, 16, 17, 19 and 22. In the second case with intrauterine retardation, severe heart malformation and fetal death, we detected deletion of 19p and additional multiple genomic deletions - the larger were del17p13 (> 4 Mbp), del17q21 (> 4 Mbp), del22q13 (> 4 Mbp). In the case with marker chromosome we established duplication of 18p (explaining the marker) and multiple additional deletions, including 1p36, 7q11, 7q21, 12q22-q24, 17p13-q21, 22q, and monosomies of chromosomes 16 and 19. Finally, more than 300 genomic aberrations (more than 80% of them were deletions), varied between several Kbp up to 5 Mbp, were detected in a fetus with holoprosencephaly. Conclusion: We conclude that chromothripsis could underlie the impaired fetal development, most probably due to the severe clastogenic effect.

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E-P01.17**De novo complex chromosomal rearrangement in a fetus with severe microcephaly**

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Introduction: Complex chromosomal rearrangements (CCRs) are structural rearrangements involving at least three breakpoints on two or more chromosomes. Such occurrences in phenotypically normal persons are rare, and the risk for abnormalities increases with the number of breaks. The majority of CCRs detected prenatally are de novo. Case Details: We present a case of fetal brain malformations including agenesis of corpus callosum, severe microcephaly, malformed vermis and distinct facial features at 24 weeks gestation. Cytogenetic analysis revealed a CCR with deletions of the long arms of chromosomes 1, 5, and 10 and at least six breakpoints, two on each chromosome: 46,XY,del(1)(q32.3q42.1),del(5)(q13.3q15),del(10)(q22q23). Chromosomal microarray (CMA) further defined the chromosomal aberrations as a loss of 1357 Kb on chromosome 1q41, a loss of 1409 Kb on chromosome 5q14.3 and a loss of 737 Kb on chromosome 10q23.31. These losses include 16 OMIM genes, 6 of which are disease-associated genes. Parental karyotype and CMA were normal. The couple chose to terminate the pregnancy. Discussion: Interstitial deletions within chromosome bands 5q14.2q15 have been described in patients with severe mental retardation, epilepsy and variable congenital brain anomalies. The severe fetal malformations were likely the result of the extensive CCR. Using both conventional karyotyping in conjunction with CMA improves our understanding of such complex rearrangements, provides an additional tool for genetic counseling and facilitates parental reproductive choices.

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E-P01.18**The chromosomal analysis of Iranian cryptorchidism infertile men**

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Introduction: Testicular descent is a process which is sometimes insufficient and results in cryptorchidism or undescended testis (UDT). Cryptorchidism is a risk factor for infertility and cancer. It has different reasons like genetic factors. Some studies have been reported about cryptorchidism boys with chromosomal anomalies. Our purpose was to investigate the association between cryptorchidism infertile men and their chromosomal constitutions in Iranian population. Materials & Methods: This research was conducted on 522 infertile men with UDT who referred to Royan institute during five years. They were selected after clinical examinations, hormonal tests, and semen and karyotype analysis. All the patients were divided into azoosperm or oligosperm, unilateral or bilateral group. Karyotype was performed using standard GTG banding technique. Results: 348 azoosperm (66.66%), 174 oligosperm (33.4%) and 45 (8.62%) chromosomal alterations were detected. Among these, seven had normal variations (1.34%) and the remaining 38 patients (7.3%) had abnormal karyotypes. Unilateral UDT were diagnosed in most of the patients with abnormal karyotypes. Klinefelter and mosaicism were the most common abnormalities, in 18 (3.44%) and 10 cases (1.91%) respectively. Furthermore, sex reversal, structural and 47, XYY syndrome were detected with lower incidence. In addition, hormonal profile showed the elevation of FSH and LH and reduction of testosterone in most of the patients with UDT. Conclusions: It seems that cryptorchidism is associated with abnormal karyotype. In case of using ART for infertility treatments, it is recommended to perform karyotype in UDT patients because they may have chromosomal abnormalities. Key-words: Cryptorchidism, Undescended Testis, Karyotype, Infertility

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E-P01.19**Parcial 18q monosomy identified prenataly as an additional finding of noninvasive prenatal testing**

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Introduction: 18q deletion syndrome is relatively common cytogenetic abnormality occurring with frequency 1 in 40 000 live births and is frequently referred to as de Grauchy syndrome. Clinical phenotype with 18q deletion is highly variable and mostly depends on the extent of genomic deletion and loss of specific genes. For the first time we report terminal deletion of long arm of chromosome 18 as an additional finding of noninvasive prenatal testing (NIPT) and thus demonstrate clinical feasibility of next generation sequencing in subchromosomal CNV detection by performing low coverage analysis. Material and methods In house NIPT test was performed in healthy 29-year old woman in 17th week of singleton pregnancy. Genomic library was prepared from cell free DNA isolated from plasma utilizing the TruSeq Nano protocol and paired-end sequencing on MiSeq (Illumina). Sequencing data were processed using in-house bioinformatic algorithm for z score calculation. Results NIPT result for trisomy 21 and 13 was negative, however with inconclusive result for chromosome 18 with abnormal z score of (minus) -16 showing large deletion in terminal part of q arm. A boy was born with severe polystigmatisation and hypotrophy. G-banded karyotype and arrayCGH were performed postnatally identifying 12 Mb deletion. Conclusion Low coverage whole genome scan utilized in NIPT can be a beneficial tool in other CNV detection besides common trisomies, given reasonable size of an event, coverage depth and fetal fraction.

M. Hýblová: A. Employment (full or part-time); Modest; Geneton. **J. Barošová:** A. Employment (full or part-time); Significant; Genet. **J. Budis:** A. Employment (full or part-time); Significant; Geneton. **L. Striešková:** None. **T. Szemes:** A. Employment (full or part-time); Significant; Geneton. **G. Minárik:** None.

E-P01.21**Analysis of maternal polymorphism of CBS gene and risk of Down syndrome offspring**

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Introduction: Down syndrome (DS) is the most common trisomy in live born with a prevalence of 1 in 1000 to 1 in 1100. Enzymes involved in one carbon and transsulfuration (1C/TS) metabolism in mothers and affected individuals with Down syndrome in altering the likelihood of birth of a child with Down syndrome. Cystathione beta synthase (CBS), located on chromosome 21 synthesises cystathione from homocysteine and serine. Trisomy for CBS may be hypothesised to decrease the availability of functional 1-C folate units. We have looked for an association of polymorphism in the CBS gene and the risk of birth of a child with Down syndrome. **MATERIALS AND METHODS:** In our study, sixty-seven DS mothers and fifty-three mothers who had no children with DS from Çukurova region of Turkey were evaluated. Genomic DNA was isolated from whole peripheral blood collected on EDTA, using salting out method. The CBS genotypes were studied by PCR-amplified products. **FINDINGS AND Results:** Among controls, the genotypes of A₁A₁ and A₁A₂ were observed in 81% and 19%, respectively, whereas the A₁A₁ and A₁A₂ genotypes were observed in 85% and 15% of case patients, respectively for CBS 844ins68 polymorphism. **Conclusion:** The results showed that the frequencies of CBS alleles (A₁ and A₂), as well as the frequencies of CBS 844ins68 genotypes (A₁A₁ and A₁A₂) do not correlate with DS pregnancies, demonstrating no difference between the case and control groups.

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E-P01.23**Functional analyses of the Fllc.*64_*66del gene variant and its potential role in fetal loss etiology**

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Introduction: Genetic variants leading to haemostatic disbalance are associated with increased fetal loss occurrence. We aimed to investigate the potential role of recently reported prothrombin (FII) c.*64_*66del gene variant in the etiology of fetal loss by case-control study approach and functional analyses.

Materials and methods: Study included 105 women with fetal loss and 154 controls. Functional assays including prothrombin activity, prothrombin plasma level, and endogenous thrombin potential were performed on plasma samples of c.*64_*66del carriers. The Cos-7 cells were transfected with two types of constructs: 1. vectors carrying wild-type and c.*64_*66del mutant full-length prothrombin cDNA; 2. vectors carrying wild-type and mutant cDNA extended with downstream regulatory element (DSE) and expression levels were determined on mRNA level by Real-time PCR and protein level by Western blot.

Results: Among women with fetal loss, three heterozygous carriers were detected, while none was recorded among controls ($0 R = 10.55$; 95%CI 0.54–206.43; $p = 0.12$). Normal prothrombin activity and plasma level were observed in all c.*64_*66del carriers. Endogenous thrombin potential was increased in carriers ($98\% \pm 6.93$) compared to control plasma (86%), but without statistically significant difference. *In vitro* analyses showed statistically significant decrease in prothrombin mRNA level for both mutant constructs ($p < 0.001$). Decrease was observed on protein level as well, but statistical significance was reached only for mutant construct without DSE ($p = 0.028$).

Conclusion: Our study indicated that FII c.*64_*66del variant is associated with decreased prothrombin gene expression which suggests its potential role in fetal loss etiology. Grant No 173008- MESTD of the Republic of Serbia.

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E-P01.24

First report of prenatal diagnosis for severe genodermatoses in Egypt

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Background: Genodermatoses are severe inherited disorders. A great success in identifying responsible genes and characterizing gene mutations paved the road for DNA-based prenatal diagnosis. Examples of severe genodermatoses candidates for prenatal diagnosis include autosomal recessive congenital ichthyosis (ARCI), Xeroderma pigmentosum (XP), Sjögren-Larsson syndrome (SLS) and Papillon-Lefèvre syndrome (PLS) where clinical severity affects span &/or quality of life hence urging prenatal diagnosis.

Materials and methods: The study included seven amniotic samples (AF) from carrier mothers descending from seven pedigrees with history of affected sibs with severe genodermatoses including; two mothers of previous ARCI cases, three XP, one SLS and one PLS. DNA was extracted from AF by QIA gene extraction kit followed by mutational screening for *XPA*, *TGM1*, *ALDH3A2* and *CTSC* genes

Results: Prenatal diagnosis was successfully performed in all cases. For the three families with history of XPA, two fetuses were found to be heterozygous carriers: one for E111X mutation and the other for R207X while the third was homozygous for T125IfsX15. For *ALDH3A2* gene, the fetus was affected for E331X nonsense mutation; for *TGM1* gene, the two fetuses were heterozygous carriers for R264W and R143H missense mutations. The fifth AF sample PLS showed homozygous wild type genotype.

Conclusion: The high incidence of consanguinity and consequently AR rare disorders combined with the lack of curative therapy, points to the importance of implementing preventive programs. Prenatal diagnosis and genetic counseling represent an important step in prevention and alleviating the burden of severe genodermatoses on the family & community.

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E-P01.25

Gonadal mosaicism: its contribution for de novo events

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A genetic abnormality in a child with healthy noncarrier parents is usually attributed to a *de novo* event during cell division (mitosis/meiosis). The recurrence of *de novo* chromosomal abnormalities can occur because of: (1) chance alone, (2) parental gonadal/ somatic-gonadal mosaicism, or (3) factors associated with an increased risk of meiotic error (in recurrent aneuploidies). When unexpected recurrences happen from apparently non-carrier parents, germ line mosaicism should be suspected. We report two cases that strongly suggest parental gonadal/ somatic-gonadal mosaicism. Case 1 refers to a healthy woman with recurrent trisomy 21 pregnancies (three). Parental karyotypes were normal but FISH analysis of oral mucosa cells and peripheral lymphocytes revealed, in the mother, low level somatic mosaicism for trisomy 21. Case 2 involves two brothers, one presenting autistic spectrum disorder and intellectual disability (ID) and the other with ID and some autistic features. aCGH analysis revealed in both siblings a *de novo* 50 kb (93,428,335–93,478,653) microdeletion in 15q26.1. Molecular testing of the parents strongly suggested a parental gonadal mosaicism. Having a previous pregnancy/ child with a chromosome abnormality is associated with an increased risk of a future chromosome alteration. In case of recurrent trisomies this risk is influenced by maternal age, that can mask other mechanisms. In recurrent structural rearrangements the more likely cause is occult parental mosaicism. Gonadal mosaicism remains an important pitfall that should be considered even during the counseling of families with *de novo* alterations, as it can seriously influence genetic risk.

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E-P01.26 Inversion Y Having Different Phenotypic Expressions at Three Brothers

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A 36 year-old patient with azospermia admitted to our out-patient clinic before IVF therapy. He had 46×, inv Y (p11q11.2) at his conventional karyotype analysis. His wife had 46,XX normal karyotype and had FVL heterozygote, MTHFR A1298C heterozygote mutations. She was normoglycemic, her hormone profile was normal and had intact tubal structure at hysterosalpingography. At the pedigree

analysis the patient's parents were first degree cousins and he had 2 sisters and four brothers. The patient had 2 brothers having the inversion Y (p11q11.2) karyotype abnormality while one of them was fertile having two children, a daughter and a son. Other married brother had no children due to female infertility factor. Remaining 2 brothers were single. Array CGH analysis of the patient revealed increases and losses at X and Y chromosomal homologue pseudoautosomal regions and a loss of 30815 kb area at Yp11.21q11.23 region. He had azospermia and at Sanger fragment analysis for Y chromosome deletion, there was loss of sY160 marker. Although his fertile brother had inversion Y (p11q11.2) at karyotype, his array analysis was normal. Herein we discussed the phenotypic expression of this chromosomal aberration.

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E-P01.28

Examination of the genotype distribution of common luteinizing hormone receptor (LHCGR) variants does not have clinical utility with regards to ovarian response to Elonva-controlled ovarian hyperstimulation

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Introduction: There are 3 common variants which impact on the receptor function of the *LHCGR* gene - ins18LQ (rs2293275), Asn291Ser (rs12470652) and Ser312Asn (rs2293275). Thus far, their combined effect on controlled ovarian hyperstimulation (COH) was not studied. Our aim was to ascertain their influence on *LHCGR* function, including their genotype combinations, in Elonva-COH

Materials and Methods: DNA samples from 48 “low”, 191 “intermediate” and 34 “high” responders (273 patients in total) were genotyped using fragment analysis for ins18LQ and TaqMan assays for Asn291Ser and Ser312-Asn variants.

Results: The genotype frequency within COH response categories was in the range 56,0%–64,7% for wt/wt, 29,4%–31,3% for wt/ins18LQ and 5,9%–6,3% for insLQ/insLQ genotypes in insLQ variant. It was 81,3%–94,1% for Asn/Asn, 5,9%–18,8% for Asn/Ser and 0%–0,5% for Ser/Ser genotypes in Asn291Ser variant, and 35,3%–35,6% for

Ser/Ser, 39,6%–55,9% for Ser/Asn and 8,8%–25,0% for Asn/Asn genotypes in Ser312Asn. No differences in genotype frequencies was found between "low", "intermediate" and "high" responders in COH ($p \geq 0,05$). Therefore, these variants cannot predict individual response to COH. Moreover, genotype combinations of studied variants did not have any significant differences, likely due to their low number within compared categories.

Conclusions: The prevalence of *LHCGR* gene variants ins18LQ, Asn291Ser and Ser312Asn is not statistically different within low, intermediate and high responders in Elonva-COH, which precludes clinical utility of their examination within this context. Supported by FNM00064203, CZ.2.16/3.1.00/24022 and NF-CZ11-PDP-3- 003-2014.

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E-P01.30

Aberrant miR-346 and miR-582-3p expression in maternal peripheral plasma, fetal cord blood and placenta as a biomarker of adverse obstetric outcomes

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MicroRNA (miRNA) was shown to regulate critical processes in fundamental cell biology and disease formation. Several miRNAs are expressed in human gestational tissue and some are identified to be associated with placenta dysfunction and abnormal pregnancy status, such as intrauterine fetal restriction (IUGR), preeclampsia, ectopic pregnancy and recurrent miscarriages (RM). In order to investigate the roles of miR-346 and miR-582-3p in the adverse events of obstetric field, we analyzed these 2 miRNAs of trio samples (maternal plasma, cord blood and placenta) obtained from pregnant women of healthy control ($n = 60$), preeclampsia ($n = 25$), preterm delivery ($n = 25$), preterm premature rupture of membrane ($n = 20$) and IUGR ($n = 20$). The expression level of miR-346 in all included adverse obstetric outcome groups was significantly higher in maternal plasma samples (all p value $< 0,05$) but significantly lower in cord blood and placenta samples (all p value $< 0,05$). Besides, the miR-582-3p level in maternal

plasma is also significantly higher in the groups of pre-eclampsia and IUGR (both $p < 0,05$) compared with normal controls. The results suggest these 2 miRNAs might play different roles in maternal and fetal systems to develop obstetric disorders, and these aberrant miRNAs expression could be biomarkers of adverse obstetric risks. Grand reference: Research Grant of National Cheng-Kung University Hospital (NCKUH- 10407001) & Ministry of Science and Technology, Republic of China (MOST 104-2314-B-006 -070 -MY3)

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E-P01.31

Association between of miR-499 Polymorphism and Recurrent Abortion

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Purpose: MicroRNAs (miRNAs) play very important roles in several physiological procedures such as successful pregnancy. Recently, findings have indicated that aberrant miRNAs gene expression and single nucleotide variation in the gene encoded miRNAs could be contributed in the pathogenesis of recurrent pregnancy loss (RPL).

Materials and Method: We were analyzing a total of 200 subjects, which were included 100 women experienced three or more recurrent miscarriages and 100 women's health with one child and without historical aberrations. *MiR-499* A >G gene polymorphism was performed by PCR-RFLP technique to assess association between the presence miRNA gene variant and with the risk of RPL. **Results:** Our results showed that the frequency of genotypes in the normal homozygote (AA), heterozygote (AG) and mutant homozygote (GG) of *miR-499* in patients and healthy women, 29%, 38%, 33% and 43%, 36%, 19%, respectively. Statistical analysis was shown significant difference genotype frequencies between cases and control groups ($p = 0,011$). **Conclusion:** These data suggested that A >G genetic variation in the *miR-499* gene encoding region could be contributed as a predisposing genetic factor to RPL.

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E-P01.32

Association of imbalance progesterone receptor-A/B ratio via + 331G/A polymorphism and Matrix

MetalloProteinases-2 and -9 expression in endometriosis

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Introduction: MMPs degrade extracellular matrix components to provide normal remodeling and contribute to pathological tissue destruction in endometriosis. It is accepted that MMPs are resistant to suppression by progesterone. The capacity of progesterone affect to gene expression is dependent on PR-A/B that imbalanced ratio may cause progesterone resistance in endometriosis. Imbalance ratio of PR-A/B via +331G/A may be able to consequence *MMP-2,-9* overexpression, which can be important in pathogenesis of endometriosis. Materials and Methods: Blood samples were recruited from 98 women undergoing laparoscopy for endometriosis and 102 healthy fertile women. Ectopic and eutopic tissues were prepared from twenty endometriosis and endometrial tissue from 20 non-endometriosis women. After DNA extraction from blood samples, genotype frequencies were determined by PCR-RFLP. RNA was extracted from tissue samples to analysis of PR-A, PR-B, *MMP-2,-9* mRNA expressions by Real-time PCR. Results: Frequency distributions of GG, GA genotypes in +331G/A polymorphism were 98.04%, 1.96% in patients and 97.96%, 2.04% in control groups respectively ($P > 0.05$). We demonstrated higher expression level of PR-B in eutopic endometrium of patients bearing GA compared to patients with GG genotypes ($P = 0.017$). Significant overexpression of *MMP-9* in ectopic samples was observed compared to control endometrial tissues, as well ($P = 0.014$). Conclusions: +331G/A seemed to have high transcriptional activity of PR-B promoter by favoring GA to GG. Our findings support alteration of PR-A/B ratio in endometriosis, which may be associated with *MMP-9* overexpression that can be important in pathogenesis of disease. This work was supported by a grant from Royan institute, Reproductive biomedicine group [91000358].

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Prenatal karyotyping in the first trimester of pregnancy in Belarus

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Introduction: First-trimester chorionic villus sampling (CVS) and second-trimester amniocentesis are widely used methods for the prenatal detection of chromosome abnormalities. We analysed identification of mosaicism cases when using both methods for cytogenetic investigation of CVS cells. Materials and Methods: Between 1996 and 2016 we collected 5309 first-trimester CVS in our department. Karyotyping was routinely performed by GTG-banding using the Trypsin-Giemsa staining technique. Cytogenetic results were obtained in 5269 first-trimester CVS (99,2%). Investigation was performed using short term culture (STC) and long term culture (LTC) techniques. Mosaicism was estimated only for cases, investigated by both methods (4466 CVS in total). Results: Mosaicism was identified in 134 cases (3,0% of CVS all taken into consideration), of which 43 (32,1%) absolute discordant karyotypes were detected by different methods (STC and LTC). A wide variety of abnormalities included quantitative abnormalities, supernumerary marker chromosomes, tetraploidy and structural rearrangements. Quantitative abnormalities of autosomes (72) and of sex chromosomes (37) were most frequently encountered mosaics. Remarkably, among the 19 occurrences of mosaic Down's syndrome in the fetus, the STC cells in 6 cases had normal karyotypes. Detection of trisomy 21 in the STC or LTC cells were always confirmed in the fetus. Both false-positive and false-negative results were obtained in trisomy 13 (12 samples in total) in STC cells. Conclusions: Decision on pregnancy continuation after detecting mosaicism requires an individual approach based on medical history and ultrasound; in some cases additional examinations are often indispensable.

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E-P01.34

Whole genome study in familial case of Mayer-Rokitansky-Kuster-Hauser syndrome

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Introduction: Mayer-Rokitansky-Kuster-Hauser syndrome (MRKH) is an anomaly of the paramesonephric (Müllerian) ducts, characterized by underdeveloped or missing uterus and variable degrees of vaginal hypoplasia, combined or not with the anomalies of ovaries and kidneys (type A and B). Its frequency in the general population is 1 in 4000–5000 live born girls and it is the etiology in 15% of cases with primary amenorrhea. Materials and methods: We have examined two sisters (age 22 and 15), both affected by Type A MRKH syndrome by using cytogenetic and array CGH analysis. The parents underwent the same testing. Results: Cytogenetic analysis showed normal karyotype. In one of the sisters, a breakage on chromosome 17q was detected. The detailed microarray analysis showed copy-number variations (CNVs) inherited from the parents and identified two copy-number variations with identical size present only in the sisters: arr(hg19)17q21.31(44171888-44351152)x3 and arr(hg19)14q11.2(19373465-19768157)x3. The first aberrant region contains KANSL1 gene, which is associated with histone modifications and acetylation. Region 17q21.31 is a hot spot for rearrangements and its CNVs are associated with different phenotypes. Conclusion: Genomic microstructural aberrations have been established in MRKH syndrome - microduplications of 17q21.31 and 14q11.2. This condition can be a result of a genomic instability and defects in transcription regulation and recombination.

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E-P01.35

Association of maternal methyltetrahydrofolate reductase gene polymorphism with neural tube defects in eastern India

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Introduction: Neural tube defects (NTDs), one of the most common forms of birth defect are highly prevalent in India (5 per 1000 birth). Studies revealed the association between genetic polymorphisms and NTDs in different part of the Globe. The aim of our study was to find out the association between MTHFR (methyltetrahydrofolate reductase) gene polymorphisms and the risk of NTDs in

eastern India. Materials and Methods: MTHFR gene was screened by bi-directional Sequencing in 46 mothers with NTD affected babies and 50 mothers with healthy babies. Results: Two SNPs: rs1801133 (C677T; ala > val) at exon 4 and rs1801131 (A1298C; glu > ala) at exon 7 were identified in our study. Our results showed that the frequency of T allele for rs1801133 was significantly higher in case compared to control (p -value = 0.013, OR = 4.92, 95% CI = 1.28–18.98) and predicted as risk allele for NTDs. Simultaneously, when we combined the variant TT genotype with the CT genotype (i.e., CT + TT), assuming a dominant genetic model, where we observed 4 fold increased risk with the combined genotype CT + TT compared with the CC genotype. On the other hand, the polymorphism rs1801131 was found non-significant to the association with NTDs in our sample. Conclusion: Though we have found the association of rs1801133 with the risk of NTDs, a large replicative study is required to establish the polymorphism for genetic screening protocol. Grant and fellowship reference: University research fund and fellowship.

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Risk assessment in non-invasive prenatal testing for aneuploidies

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Introduction Next generation sequencing (NGS) is a state of the art method used in non-invasive prenatal testing (NIPT) of common aneuploidies such as Down, Edwards or Patau syndrome. Most of the methods applied in current practice of NIPT are based on z-score statistics. The value of z-score determines the likelihood of a sample being healthy. However, such tests are known to produce false negative results occasionally. Risk assessment is a way to quantify the probability of such errors.

Material and methods We applied methods in probability and statistics to calculate risk assessment of our z-score method more precisely.

Results We incorporated the estimation of sample's fetal fraction of cfDNA in maternal plasma together with the

error of this estimation in the calculation of risk assessment of our z-score method.

Conclusion The proper calculation of risk assessment is crucial for NIPT results consulting. False positives and false negatives can lead to wrong decisions such as unnecessary amniocentesis or abortion. For this reason, it is important to know when the administered test can fail.

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E-P01.37

CFTR gene mutations in Iranian non obstructive azoospermia patients

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Background: Genetic factors cause about 10% of male infertility. Cystic fibrosis conductance regulator (CFTR) gene mutations are among relatively frequent causes of male infertility. The aim of the present study was to evaluate the effect of CFTR gene mutations in non-obstructive azoospermia. Materials and Methods: In this study, we examined the occurrence of common CFTR gene mutations in 100 non obstructive azoospermic patients as test group and 100 fertile individuals as control group. The investigated mutations were ΔF508, G542X, N1303K, W1282X, R117H and probable mutations in exons 4, 7, 10, 11, 20 and 21 in the CFTR gene, using ARMS-PCR, PCR-RFLP and SSCP methods. Results: Thirteen patients (13%) showed 406-6T>C, A120T, I148T, ΔF508, G542X mutations. None of the CFTR gene mutations were observed in the control group. Conclusion: Due to existence of CFTR mutations in DNA samples from non-obstructive azoospermia patients, the couples undergoing assisted reproductive technologies such as intra cytoplasmic sperm injection (ICSI) are advised to be screened for CFTR gene mutations.

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Non-invasive prenatal testing for screening of pregnant women with contraindication to invasive

procedure and very high risk for chromosomal abnormalities

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Background: first trimester prenatal screening is based on ultrasound and serum biomarkers and it evaluates only indirect markers of chromosomal abnormalities. Amniocentesis is a gold standard with high accuracy, however invasion is contraindicated in the risk of fetal infection. There is a dilemma for the pregnancy management for women infected by HIV or hepatitis with high risk of fetal aneuploidy. In this case, non-invasive prenatal testing (NIPT) would help to prenatal consultant manage the pregnancy, make the decision about pregnancy prolongation, but mainly to reduce anxiety and stress in pregnant women. The aims of study: discuss about implementation of clinical NIPT protocols for specific group of pregnant, namely for infected women with high risk for the aneuploidies of the fetus. Methods: at 13 week of pregnancy we used Clarigo NIPT (Multiplicom) for woman with high risk at T21 (1:4). The invasive procedure was contraindicated owing to infection by hepatitis B. Results: NIPT called negative trisomy status (trisomy evidence (T21): -3.8; Z-score: -0.3); gender: male). After genetic consulting the pregnancy was prolonged. The boy was born in term, weight 4190 g, length 53 cm and an Apgar score of 8–9; no phenotypic traits for aneuploidy has been detected when viewed by a medical geneticist. Conclusions: This case raised the discussing about implementation of NIPT to clinical protocols for pregnant women with very high risk for the chromosomal abnormalities and with contraindication of invasive procedure. Additional research are required to make full estimation for NIPT utility for discussed category of pregnant women.

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E-P01.40 Association between of P53 PEX4 Polymorphism and IVF Outcome

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Introduction: Repeated implantation failure (RIF) is the most common phenomena during IVF (In vitro fertilization) cycles treatment. Also has been reported that to achieve a successful embryo implementation, presence of nucleotide variant in the *P53* PEX4 (R72P), may be a significant effect in the endometrial receptivity of patients with IVF failure.

Materials and Methods: In case-control investigation, p53 polymorphism PEX4 (R72P) has been assessed in 200 women. We were analyzed 100 patients with IVF failure (two or more failed cycles) as well as 100 individuals with natural pregnancy as a control group, to evaluate the risk assessment between the *P53*PEX4 (R72P) polymorphism and RIF. Gene amplification was performed using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method.

Results: Our findings revealed that the frequency of the mutant genotype (PP) among control was 2% compared with 3% for RIF group ($p = 0.81$). In addition, the normal homozygote (RR) and heterozygote genotype (RP) frequencies of cases and the control group were demonstrated 47%, 50%and10%, 72%, respectively. However, the difference between the two groups was not statistically significant ($p = 0.196$).

Conclusions: Data has shown that the frequencies of p53 PEX4 (R72P) nucleotide variables were similar in the RIF and healthy group, and did not consider as a genetic predisposing factor for RIF causes.

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'When lightning strikes twice' - PGD for two genetic diagnoses

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Objectives: PGD to prevent multiple genetic disorders in the same family is a challenge for the multidisciplinary PGD team that requires perfect coordination between the genetic and IVF teams, as the total embryos suitable for transfer is lowered substantially.

Methods: a retrospective cohort of patients undergoing PGD for two or more indications at Hadassah hospital between the years 2006 to 2016. Data included genetic indications, number of IVF cycles, embryos sampled, embryos transferred (ET), pregnancy rate, and delivery rate.

Results: since 2006,859 families underwent PGD. In 15 cases (2%) there was more than one indication. Two cases (consanguineous) of 2 recessive diseases, four cases of two dominant diseases, three couples wished to conceive a healthy matched HLA sibling for a thalassemia major child for and in four cases sexing was added to another mono-genic disorder. Two couples had different indications. Number of total cycles for 12 couples was 40, 257 embryos were sampled, 42 (16%) fetuses were suitable for ET, 8 pregnancies and 7 babies deliveries were documented (one twin pregnancy). Two pregnancies are ongoing.

Conclusions: Consanguinity proportion in Israel contributes to possibility that both partners carrying more than one recessive disease. Another feature in our study population is assortative mating and the intent to avoid termination of pregnancy. There are some ethical issues regarding the creation of a stem cells donor for his sick brother, the role of sexing in the prevention of autism etc. Multidisciplinary team work is necessary in order to maximize success.

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Analysis of mRNA levels of growth factors genes in chorionic tissue and decidua at pregnancy loss during the first trimester

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Early stages of the placenta development depend on the vascular development. VEGFA and TGF β 1 are essential for development of placental vasculation during pregnancy. The aim of the study is to determine the expression

level of the growth factors genes in chorionic and decidual tissues during pregnancy. Samples of tissues were taken after surgical termination of normally progressing pregnancies (abortion for social reasons) and spontaneous abortion in 5–9 weeks of gestation. Total RNA was isolated. VEGFA and TGF β 1 expression levels were performed by RT-qPCR method. VEGFA expression in samples of both tissues in control is equal. In comparison with chorionic tissue, the expression of TGF β 1 was increased in decidua in condition of normally progressing pregnancy ($p = 0.003$). The VEGFA expression level correlated with TGF β 1 expression ($p = 0.038$). There wasn't any difference in VEGFA expression level in decidua and chorionic tissue in condition of normal pregnancy compared to spontaneous abortion. TGF β 1 expression in samples of both tissues in spontaneous abortion is equal. The positive dependence was determined for the level of mRNA VEGFA and TGF β 1 in normal pregnancy ($r = 0.6 p = 0.038$). The ratio of mRNA levels was changed in decidua ($r = -0.76 p = 0.028$) in condition of the pregnancy loss. To sum up, we showed that the change of ratio VEGFA and TGF β 1 expression levels in decidua can be associated with spontaneous abortion during the first trimester of pregnancy. This study was carried on the equipment of Center for collective use "High Technology" and supported by the federal assignment from Russian Ministry of Science and Education.

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Analysis of interaction of genes metalloproteinases, cytokines and growth factors at pregnancy loss

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The changes of structural and functional characteristics of the placenta in majority of cases cause the pregnancy loss. The backbone of this problem can be hidden in the polymorphisms of genes. The aim of this research was to investigate the interaction of SNP of such genes as MMP, cytokines and the growth factors among women with the pregnancy loss during the first trimester. The DNA from blood cells of 113 women with early pregnancy loss and 108 women with normally progressing pregnancies were examined. Polymorphisms rs1143627 *IL1 β* , rs1800795

IL6, rs1800872 and rs1800871 *IL10*, rs1800629 *TNF α* , rs1799750 *MMP1*, rs11697325 *MMP9*, rs1784423 and rs2245803 *MMP20*, rs11551797 *TIMP1*, rs2010963 and rs699947 *VEGFA*, 2073A-T *EGFR*, Arg25Pro *TGF β 1* were revealed by allele-specific PCR. We analyzed gene-gene interactions among polymorphisms using the MDR method. The research showed the certain peculiarities of genotype of women, which increase the risks of miscarriage during the first trimester. It can depend on interaction of genes MMPs, cytokines and growth factors. Genotype *MMP9* (A/A) x *EGFR* (A/T) x *IL10* (G/A) x *VEGFA* (C/A) is associated with the increasing of risks of pregnancy loss ($p = 0.025$ OR = 4,5). Another genotype *MMP9* (A/G) x *EGFR* (A/T) x *IL10* (G/A) x *VEGFA* (C/C) is associated with the reduced risk ($p = 0.005$ OR = 0.096). Investigated genes participate in structural changes of tissues and placenta formation. The break of implantation and placentation usually become the reason of the great amount of cases of pregnancy loss. This study was supported by the federal assignment from Russian Ministry of Science and Education.

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Unexpected result during preimplantation genetic diagnosis of Steinert disease

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PGD (preimplantation genetic diagnosis) is a reproductive technique used along side in vitro fertilisation (IVF) by couples at risk of passing on serious genetic condition. In case of monogenic disorder, indirect diagnosis is performed using polymorphic markers located near the gene of interest to identify the pathogenic chromosome. When possible, direct diagnosis is also performed. Here we report unexpected results in embryos at risk of Steinert disease. Steinert disease is caused by mutation in the DMPK gene located on chr. 19. Molecular analyses were performed on blastomeres of each seven embryos biopsied at J3 of development. All blastomeres of embryo 1 were disomic for chr. 19. Several blastomeres of embryo 1 showed one maternal chr. 19 and one paternal chr. 19. Interestingly, one blastomere carried one paternal chr. 19 while another blastomere carried the other paternal chr. 19. Even more unexpected, one blastomere carried

both paternal chr. 19 with no maternal chr. 19. We hypothesize a rescue of trisomy 19 to explain these discordant results. This report highlights pitfall in PGD for monogenic disorder.

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E-P01.47

A 4.29 M heterozygous loss at 8q23.1-q23.3 was associated with extreme spontaneous preterm birth

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Spontaneous preterm birth (sPTB) has become one of the most dangerous factors influencing the health of children under 5-years old, especially for the neonates. However, the pathogenesis for sPTB remains unclear. Copy number variants (CNVs) are commonly reported to be associated with pathogenesis and progression of kinds of human disorders, and the relationship between sPTB and CNV has few reported. The purpose of this study is to investigate whether chromosomal fragment variants associated with sPTB. Whole genome sequencing was carried out among 373 spontaneous preterm and 338 term delivery pregnant women, and genome-wide copy number variants were analyzed. We identified 2(0.53%) losses and 5 (1.34%) gains in preterm birth women, 3 (0.89%) losses and 8 (2.37%) gains in term delivery women, ranging from 1.06 Mb to 4.29 Mb. A heterozygous loss at 8q23.1-q23.3 was identified from a 28-week delivered woman and not found in control population. The locus contains an estrogen-responsive gene, which plays a role in the maintenance of maternal immune tolerance during pregnancy and might participate in the initiation of labor. In summary, our study identifies a CNV which associates with spontaneous preterm birth, and takes a new sight into the pathogenesis of preterm birth. Key words: preterm birth, genetics, copy number variants

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RAN Gene Polymorphism and Recurrent Pregnancy Loss

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Introduction: Unexplained recurrent pregnancy loss refers to three or more abortions before 20 weeks gestation. Exportin5, is one of the crucial molecules that has been identified in the trophoblast cells of the placenta, which was involved in the biogenesis and processing of Micro-RNA. Human embryo was produced a large number of micro RNA, which were involved in fetal development. Single nucleotide variation in *RAN* gene (rs14035;C>T) encoded could be changed quality of micro-RNA processing and effect on pregnancy outcome.

Materials and Methods: In the present study, we were included 100 women with unknown recurrent pregnancy loss as cases group and 100 women that has an at least one live child and without abortion historical, selected as a control group. After DNA extraction, we applied PCR-RFLP method for molecular genotyping of this polymorphism between both groups.

Results: Our findings revealed that the frequency of genotypes in the Wild-type homozygote (CC), heterozygote (CT) and mutant homozygote (TT) of *RAN* in patients and healthy women, 71%, 25%, 4% and 71%, 20%, 6%, respectively. Statistical analysis was not shown significant difference genotype frequencies between cases and control groups ($p = 0.451$). **Conclusions:** The present study revealed that *RAN* gene polymorphism cannot be a predisposing factor for recurrent pregnancy loss in the Iranian population.

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down regulation of Ca²⁺ ion channel coding genes in endometrium of women with recurrent implantation failure

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Introduction: The critical role of ion channels on reproduction have been reported. During implantation, Ca²⁺ plays a major role when uterine undergoes decidualization and fluid absorption. Three genes code for the T-type

voltage-gated calcium channels that encoded by *CACNA1G*, *CACNA1H* and *CACNA1I* are expressing in the epithelial cells of a numerous kinds of tissues, such as the human endometrium. Materials and methods: Consent was obtained from patients according local ethical approval. Endometrial injury obtained from 20 infertile women with recurrent implantation failure (RIF) between 22–35 years old in 19th–24th days of menstrual cycle (window of implantation) by pipelle. Also, endometrial biopsy obtained from nine 22–35 years old fertile donor women in a cycle before ovarian stimulation as control through window of implantation by pipelle. Relative expression of *CACNA1G*, *CACNA1H* and *CACNA1I* in endometrial samples of fertile and RIF women were evaluated quantitatively by real-time PCR. Results: The data showed mRNA expression of *CACNA1H* gene decreased significantly in endometrium of RIF patients vs. fertile group. (*P*-Value ≤ 0.01) Whiles, lower mRNA expression of *CACNA1G* and increased expression of *CACNA1I* in endometrium of RIF patients vs. fertile group but these alterations were not significant. Conclusions: These data reveled association of altered ion channel levels and recurrent implantation failure.

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E-P01.50

Contribution increase of JAK2 D620K and C618W variants and reduction of JAK2 V617F variant and associated lack of D661Y and Y640F variants in STAT3 gene in Iranian women with miscarriage

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Recurrent miscarriage (RM) is the occurrence of repeated pregnancies that end in miscarriage of the fetus before 20 weeks of gestation. Considering the significant proliferative functions of JAK2 gene provides instructions for making a protein that promotes the growth and division (proliferation) of cells and The STAT3 gene provide instructions for making proteins that are part of essential

chemical signaling pathways within cells. The STAT3 protein transmits signals for the maturation of immune system cells. We decided to investigate the correlations between miscarriage in Iranian women and JAK2 V617F/ C618W/D620K, STAT3 Y640F /D661Y/T632I/S636F/ V637M variants. DNA was extracted from blood samples of 24 unrelated Iranian women with three or more unexplained pregnancy losses occurring in the first trimester as well as Forty eight healthy subjects as control group. Genomic DNA was used to detect somatic mutations in JAK2 and STAT3 genes. The PCR products were detected by capillary electrophoresis (CE) and subsequently extracted for sequencing. *JAK2 V617F mutation was identified in 6 (28.4%) of 48control. All variants were confirmed by sequencing. Sensitivity studies showed JAK2 V617F (P < 0.05) was associated with decrease IRM (idiopathic recurrent miscarriage) risk in Iranian women and STAT3 Y640F (P > 0.05) and D661Y (P > 0.05) variants were not associated with RM.* Our phenotype-genotypic association analysis indicated that there was insufficient evidence to demonstrate an association between JAK2 D620K, V617F variants and the risk of RM. D620K and C618W variations increase RM risk But V617Fvariation decreases RM risk in patients with three or more miscarriages.

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Negative Effect of the *Dicer* Gene Polymorphism and Recurrent Miscarriage

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Introduction: Unexplained recurrent pregnancy loss refers to three or more abortions before 20 weeks gestation that approximately occurs in about one percent of the total number of couples. *Dicer* is an important molecule that has been characterized in the micro-RNA biogenesis procedures. This molecule plays crucial roles in the maturation of micro-RNA and is also involved in DNA damage response. Single nucleotide polymorphism in the *Dicer* (rs3742330; A > G) encoded gene might be to produce the dysfunctional enzyme which was involved in the implantation process and leads to the pregnancy loss.

Materials and Methods: We were designed a case-control investigation in the 100 subjects with unexplained recurrent pregnancy loss and 100 women with at least one child and without historical abortion as a control group.

After DNA extraction, we used PCR-RFLP technique, in order to clarify the association between *Dicer* gene polymorphism and recurrent pregnancy loss.

Results: Our findings revealed that the frequency of genotypes in the Wild-type homozygote (AA), heterozygote (AG) and mutant homozygote (GG) of *Dicer* in patients and healthy women, 75%, 25%, 0% and 70%, 30%, 0%, respectively. Statistical analysis was not shown significant difference genotype frequencies between cases and control groups ($p = 0.430$).

Conclusions: The present study disclosed that polymorphism of the *Dicer* gene cannot be considered as a predisposing factor in unexplained recurrent pregnancy loss.

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quantitative fluorescence polymerase chain reaction, rapid method to detect rare 48, XYYY syndrome; a case report

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Introduction: In this case report, we discuss the rare sex chromosomal abnormality of 48, XYYY syndrome, which shares some physical features similar to that of Klinefelter syndrome (47, XXY). Approximately 20 cases of 48, XYYY have been reported to date. Materials and Methods: In this report, a patient who was diagnosed with 48, XYYY syndrome with clinical evaluation and cytogenetic analysis is presented. Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR) was used for rapid sex chromosomal aneuploidy Results: Karyotype analysis indicated a chromosomal composition of 48, XYYY which confirmed the QF-PCR result. the results of QF-PCR indicated an extra pair of Y chromosomes in contrast to the normal male Conclusions: Whilst being a slow procedure, the classical method of detecting extra chromosomes, karyotyping produces reliable results. In contrast to karyotyping, new modern methods of molecular cytogenetics such as QF-PCR are rapid procedures with high accuracy to detect possible chromosomal aneuploidy. QF-PCR is based on polymorph-none- polymorph STRs which indicate the total number of chromosomes. The other advantage in QF-PCR is related to allelic and none allelic STRs which may be useful for distinguishing the origin of a chromosome. Finally, one of the main complaints at presentation in our

patient was infertility, as Klinefelter males (47,XXY) are often infertile or have reduced fertility, it may be worth considering the role of increasing numbers of Y chromosomes in infertility.

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An atypical translocation 46,XY,t(4;13)(q12;p12) with a peculiar length of satellite stalks

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Abstract: Apparently balanced reciprocal translocations are a common type of chromosome rearrangements with an estimate incidence range from about 1 in 500 to 1 in 625 human newborns. Rearrangements were found both in clinically unaffected individuals and patients with phenotypic abnormalities. Most are inherited, but approximately one in five are de novo events and introduce a risk of abnormal phenotype in 6.1% of prenatal genetic counseling. Methods: In the present case, we reported the analysis results of prenatal diagnosis using G-banded karyotype, nuclear organizer regions staining and CGH array. Initial chromosome analysis of amniotic fluid samples was performed by standard G-banding. The karyotype analysis was extended on both parents culturing peripheral blood in order to elucidate the chromosome rearrangements in question. Results: The cytogenetic study revealed an atypical translocation 46,XY,t(4;13)(q12;p12) with a peculiar length of satellite stalks of chromosome 13. This uncommon condition was highlighted using Ag/NOR staining. After informed consent analysis was extended to the parents. Parental origins of the translocation was excluded by the cytogenetic analysis on both parents defining this to be a de novo rearrangement. Finally using array CGH was confirmed the absence of genetic material lack in proband and the nonappearance alterations in parents corroborating the evidence of de novo balanced translocation. Conclusion: Although balanced translocations are usually associated with normal phenotypes, abnormalities are attributed to an imbalance of dosage sensitive genes that change spatial location and carriers increased risk of reproductive failure. Our results should be interpreted with caution and required a careful follow up.

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E-P01.54
The significant role of STR markers in complex diagnosis of PND or PGD of β thalassemia

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β -thalassemia is the most common hematological disorder worldwide. Because of the high carrier rate of beta globin gene mutations in Iran, prenatal diagnosis (PNDs) or Pre-implantation Genetic Diagnosis (PGD) are the favored procedures to prevent birth of new β -thalassemia cases. Haplotyping, using STRs (Short Tandem Repeat) markers linked to β -globin gene, would add accuracy to any PND and is essential for every PGD for β -thalassemia. In this research, we identified several novel STR markers linked to the β -globin gene, with the aim of increasing the specificity and sensitivity of the diagnosis. Allele frequency and heterozygosity assessment of these STR markers were studied on 100 unrelated healthy Iranian individuals. In total, 97 alleles were detected for ten STR loci. The observed range of allele frequencies was from 0.7% to 38%. The frequencies of genotype distributions for all ten STRs were found to be $P \geq 0.1876$ which is in agreement with the Hardy-Weinberg equilibrium. Genotyping of all couples were determined by ARMS, MLPA or Sanger sequencing methods. Linkage analysis was used to confirm the results of molecular findings. Also, PGD was applied for 14 couples at risk of transmitting β -thalassemia to their offspring. We found that these markers can easily be applied to PGD and it would be very useful to be incorporated in some PND cases of β -thalassemia.

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E-P01.55

A homozygous deletion of exon 7 in LIFR gene in a neonatal case of Stuve-Wiedemann Syndrome

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Introduction: Stuve-Wiedemann syndrome (STWS; OMIM #610559) is a rare autosomal recessive bent-bone dysplasia characterized by bowed long bones, respiratory distress, feeding difficulties, and hyperthermic episodes. It was thought to be a lethal condition but there are reports describing patients who survive. We reported 2 sibs, with neonatal STWS characteristics, offspring of first-cousin parents heterozygous for deletion of LIFR exon 7. Variant doesn't describe to our knowledge at this time.

Materials and Methods: Male born at 41 weeks gestation after induced labour for oligohydramnios and with suspicion of skeletal dysplasia in uterus. Presenting at birth: facial anomalies, bowing of long bones, limited mobility of elbows, camptodactyly, respiratory distress and pulmonary hypertension, feeding difficulties and hyperthermic episodes. Parents, first cousin marriage, with positive family history of sib recurrence with a previous 8-month-old son dead with bone dysplasia and acute exacerbation of chronic shortness by bronchiolitis, refused prenatal diagnosis.

Results: Parental consanguinity, sib recurrence and clinical findings suggested STWS syndrome. Sequence analysis of LIFR gen was performed and showed a homozygous exon 7 deletion [r.562_736del];[r.562_736del]. Heterozygous carrier status of both parents was confirmed.

Conclusions: Patient survives at 18 months with an improvement in their ability to swallow and regulate breathing as literature describes. The analysis supported the clinical homogeneity of SWS, despite genetic heterogeneity. Although STWS is a rare condition and the prognosis is poor, management strategies could increase long-term survival. Unfortunately, at the moment the family doesn't accept any genetic advice.

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E-P01.56**Null allele detected in the context of paternity by DNA fingerprinting**

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Introduction: Paternity tests enable to establish the biological relationship between a child and his alleged father. Today, this test is based on DNA fingerprinting using mainly short tandem repeats (STRs) markers. To be the father, an individual must share with the child at least one of the two alleles of each marker studied. We report in this paper a case of null allele genetically confirmed in the context of paternity by genetic fingerprint.

Observation: We performed a genetic study of three members: a mother, her baby, and a supposed father. This study involved the analysis of 15 STRs markers by "PowerPlex 16 System" kit.

Results: Genetic analyzes were performed under the same technical conditions and showed that the VWA marker presented abnormal transmission between the infant and his mother for a null allele. This hypothesis was confirmed by the study of a second kit that enabled to view this allele and showed that it is normally transmitted to the infant.

Conclusion: The kits used in the context of paternity by genetic fingerprint use different primers in the amplification of markers and the detection of alleles, which is more or less the specific polymorphisms in each population. So these kits are seen as complementary and should be used concomitantly in the identification by DNA fingerprinting studies. In this study, we have confirmed this situation by molecular genetics tools. However, their clinical and biological implications remain unknown.

W. Manoubi: None. **A. Mili:** None. **A. Msakni:** None. **A. Saad:** None. **M. Gribaa:** None.

E-P01.57**Prenatal diagnosis of atypical Turner syndrome: case report of idic(Xp) with multiplication of Xist locus**

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Turner syndrome (TS) is characterized by absence of X chromosome in woman. Different chromosomal anomalies

are associated to TS: monosomy X (homogeneous or mosaic) and structural X chromosome anomalies (deletion, Xq isochromosome...). Isochromosome Xp is rare, only about ten cases are reported to date. Each of these patients carry the dicentric isochromosome Xp, including a variable part of Xq chromosome, in a mosaic state. We report the case of a prenatally diagnosed girl who carries a complex X-chromosome rearrangement in a mosaic state. Amniocentesis was performed because of intrauterine growth retardation, aortic hypoplasia and atypical serum markers. Karyotyping showed a mosaic 45,X/46,XX with additional X chromosome which seems to have abnormal structure. Array-CGH showed revealed presence of an idic(X)(pter->q12::q12->pter) with multiplication of Xq13 region (containing Xist locus). These anomalies were confirmed by FISH. After birth, evolution was marked by growth retardation and persistence of isthmic aortic hypoplasia. Chromosomal abnormalities were confirmed by cytogenetic analyses (FISH, ACPA, semi-quantitative PCR). At the age of 1 year, psychomotor development occurs normally. Prenatal diagnosis of TS can be done fortuitously or following echographic abnormalities. In these cases, discussion about termination of pregnancy is difficult because psychomotor development is usually normal outside the cases of abnormal X with Xist locus loss. For our patient, no case with Xist locus multiplication has been reported to date. Moreover, duplications of Xq12.3q13 and Xq13q21 are associated with psychomotor delay and autism in boys, phenotype is normal in girls. These data make genetic counseling in this sensitive situation.

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E-P01.59**Association of coagulation factors genes polymorphism with uncompleted carriage**

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Introduction. The hemostatic changes in blood system of the mother occur in all pregnant women as a result of adaptation of the organism to the condition of pregnancy, most of them towards changes in coagulation system. Previous studies suggest that mutation/polymorphism of genes encoding coagulation factors may increase a risk of

uncompleted carriage but this data are very controversial. So, the aim of this study was to determine the association of SNPs in FII, FV, FXI and FII genes with uncompleted carriage in women of Tatar ethnicity (Russia). Materials and methods. Genotyping of FII (rs1799963), FV (rs6025), FXI (rs2289252), FXII (rs1801020) genes mutation/polymorphism was performed by RT-PCR with TaqMan probes in 92 women with insufficient carriage (UC) and 161 women with uncomplicated physiological pregnancy (UPP). Statistical analysis done with packet program RStudio. Results. We observed no significant difference of genotype frequencies in UC and UPP women for commonly studied in complicated pregnancies Leiden mutation (coagulation factor FV), prothrombin FII gene and *C46T* FXII gene ($p > 0.05$). However increased genotype CT frequency for FXI gene *C22771T* polymorphism (rs2289252, located in intron region) was determined in women with UC (OR 3.19, 95%CI 1.678 - 6.0815) but studied polymorphism didn't influence changes in hemostatic parameters such as platelet counter, PT and APTT ($p > 0.05$). Conclusion. Hemostasis instability during pregnancy may cause spontaneous abortion but its effect depends on many factors including the genetic polymorphism that varies among different ethnicity that must be taken into account in genetic counseling practices.

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E-P01.60

Correlation of VEGFA polymorphism + 405G/C with reproductive failure in Bulgarian patients

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Introduction: Approximately one of six couples in reproductive age worldwide experience different difficulties in conceiving. The successful embryo implantation depends on trophoblast proliferation, migration and invasion in the endometrium. This is mediated by locally produced molecular factors and hormones. Vascular Endothelial Growth Factor (VEGF) is one of these factors that play pivotal role in human angiogenesis and embryogenesis. Materials and

methods: Blood samples were collected from control women with natural conceived successful pregnancy ($n = 21$) and patients with reproductive failure ($n = 41$): RIF - Recurrent Implantation Failures, endometriosis and Recurrent Spontaneous Miscarriages. Genomic DNA was isolated. VEGFA + 405G/C genotyping was performed by PCR-RFLP using BsmFI restrictionase. Real Time PCR and Sanger capillary sequencing were used for validation. Results: The C allelic frequency was higher in patients group compared to controls (53.6% vs 24%, OR = 3.68, 95% CI, $p < 0.002$). In addition, we detected statistically significant incidence of the + 405 C/C genotypes in women from the patient group than in control group (37% vs 4%, OR = 16.2, 95% CI, $p < 0.02$ when calculating against GG genotype). The results of the present study revealed association between the VEGFA + 405 C/C genotype and the risk of development of endometriosis and spontaneous miscarriages. In the RIF the results did not reveal such correlation. Conclusion: This test could be offered to patients with presented indications and special attention to be paid on their clinical management. The study group should be expanded and include more genetic polymorphisms in association with the mentioned reproductive pathologies.

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E-P01.61

Prevalence of HIV, HBV, HCV and risk factors in pregnant or puerperal women in Angola

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Globally, about 34 million people are infected with HIV, 240 million are chronically infected with HBV and between 130–150 million with chronic hepatitis C infection. Despite the worldwide distribution, the WHO estimates that the prevalence of such viral agents is highest in sub-Saharan countries. However, little is known about the prevalence, genetic diversity and rate of mother to child transmission in Angola. The aim of this study was to describe the prevalence of HIV, HBV and HCV in pregnant or puerperal women who attended prenatal clinics or maternities in the capital city and other five regions of the country to gather necessary information for further research. 429 Blood samples were initially submitted to the anti-HCV antibody (Ab), anti-human immunodeficiency virus (HIV) Ab, and hepatitis B surface antigen (HBsAg) tests. While the demographic data specifies that 48.5% participants were single, the mean age was 24.9

and up to 77.9% had educational levels equal or inferior to high school and 49% had no formal employment; the epidemiologic results indicated that 4% of women were positive for HIV, 8% for HBV and 3% for HCV. Statistical analysis showed that the variables were positively associated with age, the level of education, marital status, occupation, and multiple partners. The prevalence of infection among the women suggests a concerning rate of vertical transmission and high exposure to risk factors. Initiatives for determination of genetic variability and viral load for effective management of these infections along with community education, early diagnosis and prevention of transmission are urgently needed.

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E-P01.64

Detection of Y microdeletion in Bosnian males with fertility disorders

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Introduction: Genetic factors related to male infertility are chromosomal abnormalities, gene mutations, and microdeletions on Y chromosome, which are detected in about 15% of all cases of male infertility. The aim of this study was to identify the site specific microdeletions of Y chromosome in idiopathic azoospermic and oligozoospermic cases from Bosnian population. Materials and methods: During the 4 year period (31/01/2013 to 31/01/2017), 42 patient samples were received by the Human Genetics Laboratory, Clinical Center of the University of Sarajevo, referred for suspected male infertility for the detection of Y chromosomal microdeletion. Both karyotype and PCR analysis for Y microdeletion (GML Y Chromosome Microdeletion Detection System Kit, Altendorf, Switzerland) were performed for each patient. Results: In the analyzed period, 2% of patients had either chromosomal or Y deletion defect. In 1.2% of patients Y microdeletion was detected ($n = 5$). Most patients had the complete deletion of AZFd region ($n = 4$), followed by partial AZFc deletion ($n = 4$), and complete AZFa or AZFb deletions ($n = 2$ patients each). Additionally, four patients had chromosomal abnormalities including translocations of autosomal chromosomes ($n = 2$), an insertion of heterochromatin on chromosome 17q21 ($n = 1$) and Klinefelter syndrome ($n = 1$). Conclusion: Since the detection of Y microdeletion was established 4 years ago in Bosnia and Herzegovina, we evaluated the incidence and significance of chromosomal and Y-microdeletion defects in

order to create future guidelines for genetic diagnostic of male infertility.

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E-P01.65

Tetrasomy Xq in a Primary Infertile Case

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Structural abnormalities of the X chromosome are usually associated with the abnormalities resulting in primary amenorrhea and infertility. The majority of these cases are caused by the deletion of p and q arms of X chromosome. Among those anomalies the number of cases related to Xq partial tetrasomy is very small. Here we present a 25-year-old female case who was referred because of primary infertility and amenorrhea. There was no consanguinity between her parents and she was the only healthy sister. There was no significant finding neither in her family history nor in the physical examination. Molecular genetic analysis of FMR1 gene was normal. Peripheral blood karyotype analysis revealed 46,XX,dup(Xq) and her parental karyotypes were normal. The CytoScan® Optima Suite (Affymetrix) microarray data revealed four copy of Xq13.2; q27.2 region (arr[hg19] Xq13.2q27.2(72,332,065–140,514,863)x4). Nearly 68 Mb region on X chromosome was predicted to be tetrasomic. To the best of our knowledge this is the second case presenting partial tetrasomy in Xq. The X chromosome regions responsible for the abnormal phenotypes are poorly understood. This case may explain the phenotypic effects of X inactivation and X chromosome abnormalities.

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E-P01.66

Genetics of Pre-Term Birth suggest a role of a WNT pathway gene in Spontaneous preterm birth

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Introduction: Preterm birth defined as birth at less than 37 weeks of gestation, is the leading global cause of mortality in children under five years of age. A substantial body of evidence suggests the contribution of genetic factors in birth timing and the risk of preterm birth, largely residing in the maternal genome. Materials and methods: A genome wide association study and functional analysis conducted by our group, suggest that WNT4 is associated with gestational length. Pathway analysis of the WNT pathway was further carried in a cohort of 86 Danish sister pairs with spontaneous preterm birth. Results: Rare variants found in the 3' and 5' untranslated regions of a WNT pathway gene and predicted to cause five new binding sites for microRNAs, were identified in the Danish sister pairs. The gene is highly evolutionary conserved and expressed in cells secreting WNT. It was shown to have an important role in lung development and angiogenesis in mice, but its role in placental angiogenesis was not directly studied. Conclusion: The WNT pathway gene may have a role in preterm birth, mainly mediated through its gene expression regulatory elements. Its function could have a role in placental angiogenesis.

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E-P01.67

Experience of NIPT testing in United Medix laboratories: 965 cases in 2015–2016

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During 2015–2016 965 non-invasive prenatal tests (NIPT) were performed using verifi® Prenatal test by Illumina. The test detects trisomies 13, 18, 21, 9 and 16 and X and Y chromosomes (807/965) and when requested, also six common microdeletions (158/965). All samples that arrived in time (≤ 5 days) were successfully performed (100%). The indications were either increased risk in first or second trimester screening test, maternal age, chromosome abnormality in previous pregnancy, abnormal ultrasound finding, worry or missed first trimester screening test. Majority of the samples (909/965) had normal result (417/XX and 492/XY). Abnormal result was detected in 56 samples (5,8%): 27 trisomy 21, 11 trisomy 18, three trisomy 16, three trisomy 13 and one with both 13 and 18 monosomy, six X monosomy, three XXX and one XXY

and XYY cases. Abnormal results were confirmed with invasive test in 34 out of 56. 9 clear false positive (FP) results were detected (0,93%): one trisomy 16 case, three trisomy 18 cases, three chromosome X monosomy cases and simultaneous monosomy 13 and 18 case. One XYY case turned out to contain XXY sex chromosomes. Three cases with triple X in NIPT test will be studied after the deliveries and in two of these the mother had 47,XXX karyotype. All FP cases are known pitfalls of the NIPT test. None clear false negatives (FN) were detected. To conclude, NIPT test offers safe and reliable screening test choice. Proper precounseling for all patients, and invasive test to confirm abnormal results are extremely important.

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

E-P02.02

Novel mutation in *SIX1* in a girl with branchiootic syndrome

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Branchiootorenal spectrum disorders comprise branchiootic (BOS) and branchiootorenal (BOR) syndromes, both characterized by second branchial arch anomalies that may include auricular or preauricular defects and hearing loss (conductive, sensorineural, or mixed) and renal anomalies in BOR. Three genes *EYA1* (40% of cases), *SIX5* (5%) and *SIX1* (2.3%) have been associated with BOS/BOR. According to previous reports most of the patients with mutations in *SIX1* do not have categorical renal manifestations. We report a 2 year old girl with bilateral sensorineural hearing loss, cochlear agenesis, cleft palate, facial asymmetry and hypermobility. Spine X-ray and abdominal ecography were normal. Given the suspicion for diagnosis of BOS, genes in the spectrum of BOR disorders were studied. Sanger sequencing of *SIX1* were performed in whole blood DNA and resulted in the detection of a novel

heterozygous transition c.522 C > G in exon 1, predicting an Asn > Lys substitution in residue 174. This variant is located on the helix 9 of the homeodomain part of the protein and is evolutionary conserved. The affected aminoacid N174 corresponds to the N51 residue of the α 3 in the canonical homeodomain, being one of the critical aminoacids in the major-groove interaction in HD-DNA recognition and its alteration reduces the binding affinity. The change was not found reported neither in pathogenic variants nor in population databases. *In silico* analysis by several predictors classified it as pathogenic. Our findings in a patient with BOS and without renal involvement support previous reports of SIX1 mutations to establish a more accurate phenotype-genotype correlation.

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E-P02.03

Genetic analysis of CHST6 and TGFB1 in Turkish patients with corneal dystrophies: Five novel variations in CHST6

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Introduction: To identify pathogenic variations in *carbohydrate sulfotransferase 6* (*CHST6*) and *transforming growth factor, beta-induced* (*TGFB1*) genes in Turkish patients with corneal dystrophy (CD).

Methods: In this study, patients with macular corneal dystrophy (MCD; n = 18), granular corneal dystrophy type 1 (GCD1; n = 12), and lattice corneal dystrophy type 1 (LCD1; n = 4), as well as 50 healthy controls, were subjected to clinical and genetic examinations. The level of antigenic keratan sulfate (AgKS) in the serum samples of patients with MCD was determined with enzyme-linked immunosorbent assay (ELISA). Variations were analyzed with DNA sequencing in the coding region of *CHST6* in MCD and exons 4 and 12 in *TGFB1* in LCD1 and GCD1.

Results: The previously reported R555W mutation in *TGFB1* was detected in GCD1, and the R124C mutation in *TGFB1* was detected in LCD1. Serum AgKS levels indicated that 12 patients with MCD were in subgroup I, and five patients with MCD were in subgroup II. In patients with MCD, three previously reported missense variations (c. 1 A > T, c.738 C > G, and c.631 C > T), three novel missense

variations (c.164 T > C, c.526G > A, c. 610 C > T), two novel frameshift variations (c.894_895 insG and c. 462_463 delGC) were detected and no genetic variation was detected for three patients with MCD type II.

Conclusions: This is the first molecular analysis of *TGFB1* and *CHST6* in Turkish patients with different types of CD. Five novel likely pathogenic variations in *CHST6* were reported in MCD. This study was funded by TUBITAK (Grant no: 114S126)

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E-P02.05

Whole exome sequencing reveals NM_005267.4: c.130G > A mutation in GJA8 gene in a large Mexican family with autosomal dominant cataract

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Cataract is the cause of half of blindness and one third of visual impairment worldwide. Congenital cataract is an entity clinically and genetically heterogeneous with high penetrance and represents 10% of a treatable cause of childhood blindness. Thirty percent of the cataracts are hereditary with the nonsyndromic autosomal dominant form. In the present study, we analyzed a large Mexican family affected by autosomal dominant cataract in four generations through whole exome and identified the NM_005267.4:c.130 G > A (p.Val44Met) mutation on *GJA8* gene. Missense c.130 G > A mutation has not been previously reported. This is the first Mexican family with congenital cataract associated to a p.Val44Met mutation. The variant c.130 G > A (p.Val44Met), is in heterozygosity in the *GJA8* gene. Mutations in this gene have been associated with cataracts of multiple types with autosomal dominant inheritance pattern. To confirm the pathogenicity of variant c.130 G > A P.Val44Met in the *GJA8* gene, we analyzed affected and non-affected members of the family and 100 normal controls. This variant cosegregated exclusively with affected members, so this result could be compatible with the congenital cataract described in the family. This data shows evidence of heterogeneity genetic in autosomal dominant cataract in the *GJA8* gene.

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E-P02.06

Three novel CYP1B1 mutations (p.L480P, p.S476P, p.R175P) in Primary Congenital Glaucoma Cases residing in Eastern Iran

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This study was aimed to screen 27 familial cases of PCG for CYP1B1, to identify and determine common mutations, and to understand its penetrance and prevalence in the Eastern provinces of Iran. Methods: Detailed family histories up to three generations were taken, and pedigree charts were constructed. Genomic DNA was extracted from peripheral leukocytes. Primers were designed for the two coding exons of the CYP1B1 gene and the amplified products were sequenced in both forward and reverse directions with the same primers as used in the PCRs. Results: Comprehensive analysis sequencing revealed heterogeneity of mutations in these patients. About 63 percent had mutations in the *CYP1B1* gene. In this study, 10 specific mutations associated with disease phenotypes found. Six missense p. R368H, p.E229K, p.R390C, p.V364M, p. F445I, p.G61E and a deletion mutation c.1504_1504delA were previously reported as 3 missense mutations, p.L480p, p.S476P and p.R175P, were novel. G61E mutation was identified as the most common mutation in approximately 47% of cases. We also observed that one of the patients was homozygous for the mutation E229K, and R390C (tetra-allelic). Conclusion: Mutations in *CYP1B1* are a major cause for PCG in our patients. Identifying mutations in subjects at risk of developing glaucoma, particularly among relatives of PCG patients, is of clinical relevance. These developments may help in reducing the disease frequency in familial cases. Such studies will be of benefit in the identification of pathogenic mutations in different populations and will enable us to develop simple and rapid diagnostic tests for analyzing such cases.

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E-P02.07

First results of genetic testing of non syndromic hearing impairment in patients from Serbia- case report of family with multiple affected members

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Hearing impairment is the most common sensorineural disorder with the incidence estimated to be 1:700–1000 newborns. Mutations in *GJB2* (gap junction beta 2) gene are the major cause of autosomal recessive non syndromic hearing impairment (ARNSHI). The most prevalent mutation in many populations is a point mutation in protein coding sequence of *GJB2* gene, known as a c.35delG. In Caucasians, this mutation represents the most common cause of ARNSHI, with frequency of 70% and carrier rate of 1–3%. In 2016. we started with long-term study introducing genetic testing for the presence of c.35delG mutation in Serbian patients suffering from ARNSHI. Final goal is to define, for the first time, frequencies and distribution of mutations in *GJB2* and *GJB6* genes in Serbian population. During one year period analysis were done in 30 patients (60 samples including family members). Here we present a family whose members had clinical diagnosis of bilateral sensorineural hearing impairment with range severe to profound, which was compatible with ARNSHI. DNA analysis was performed using partially modified PCR-ARMS assay for direct detection of c.35delG. All patients with ARNSHI were homozygotes for c.35delG mutation (two brothers, their wives and children). Healthy members (parents) were heterozygote carriers. Appropriate genetic counseling was offered to the family. This is the first report demonstrating the genetic cause of clinical deafness in Serbian patients with ARNSHI. In this paper authors will discuss details of genetic analysis, possible genotype/phenotype association and phenotypic plasticity in probands.

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E-P02.08

Prevalence and scale of *GJB2*, *GJB6* & *SLC26A4* mutations associated with nonsyndromic hearing loss in the eastern part of India

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Introduction: Hearing impairment is one of the most common neurosensory disorders and is genetically heterogeneous. In spite of this heterogeneity, mutations in the GJB2, GJB6 and SLC26A4 are the major contributor. Only few studies were conducted on the genetic alteration of these genes in different demographic region of India but never focused on the eastern part of this country. The present study, first time aimed to characterize the mutation spectrum of GJB2, GJB6 and SLC26A4 gene in hearing impaired patients of West Bengal state, India. **Materials and Methods:** 215 congenital nonsyndromic hearing impaired probands were genetically evaluated by direct sequencing. Radiological diagnosis was performed in patients with SLC26A4 mutations by temporal bone CT scan. **Results:** Of 215 patients investigated, 6.97% were diagnosed to carry biallelic mutations while in 4.65% had only one mutant allele. Six mutations (M1V, T8M, W24X, W77X, V84L and E147K) were identified; W24X was the most frequent, accounting for 71.05% of the mutant allele. Mutations in GJB6 were not identified in our study. Further, no patients harbored bi allelic SLC26A4 mutations or the bilateral Enlarged Vestibular Aqueduct in our study. **Conclusions:** The mutation profile of GJB2 in our study is distinct from other parts of India, suggesting that the mutation spectrum and frequency of this gene alters with ethnicity and geographical origin. The absence of GJB6 mutations and low frequency of SLC26A4 mutations pointing that additional genetic factor may contribute to the Non Syndromic Hearing Loss in our population. Grant reference: DBT, WB, India [106-BT (Estt.)/RD-20/11].

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miRNA expressions in pre,per and post lingual hearing loss

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Introduction: MicroRNAs (miRNAs) are small non-coding RNA molecules that control post-transcriptional gene expressions in various cellular processes including proliferation, differentiation, development and apoptosis. Hearing is complex process of coordinated units of ear including gap junctions, ion channels, hairy cells and several signalling and motor proteins. Any cease in development of those units would result with sensorineural hearing loss. Here, we aimed to investigate the miRNA expressions in hearing loss.

Material and Method: 11 miRNAs were evaluated in serum samples of 38 patients with pre-lingual(n = 19), per-lingual (n = 4), post-lingual (n = 15) hearing loss and 12 controls. miRNAs were analysed by real time PCR (RotorGene Q, Qiagen) method using miScript miRNA primer assays and SYBR Green PCR kits (Qiagen) according to manufacturer instructions.

Results: Table. miRNA expression fold regulations within pre-per-post lingual hearing loss patients compared to controls.

Discussion/Conclusions: Significant decreased expressions of apoptosis and angiogenesis controlling miRNAs (miR-126 miR-222, miR-92) in patients might be the reason of hearing loss through over expression of connexins at supportive cells and connective tissue cells in the inner cochlea by leading ultra rapid exchange of ions.

Fold Change (comparing to control group)

Pre lingual		Per lingual		Post lingual	
Fold Regulation	95% CI	p-value	Fold Regulation	95% CI	p-value
MIR17	1,2793	(0.00001, 2.62)0,544	3,529	(0.00001, 8.43) 0,355	1,965
MIR92	-34,88	(0.00001, 0.07) 0.000014	-42,05	(0.00001, 0.14) 0,922	-63,86
MIR126	-51,51	(0.00001, 0.06) 0,002	-19,47	(0.00001, 0.27) 0,246	-36,87
MIR146a	-4,311	(0.00001, 0.60)0,471	1,640	(0.00001, 11.66)0,070	-7,304
MIR181b	-2,870	(0.00001, 1.03)0,417	9,167	(0.00001, 69.91)0,069	-1,682
MIR183	-4,627	(0.00001, 0.45)0,595	3,495	(0.00001, 24.04)0,069	-3,534
MIR210	-1,395	(0.00001, 1.50)0,742	-1,958	(0.00001, 1.50) 0,676	-1,507
MIR222	-30,45	(0.00001, 0.08) 0.00067	-6,136	(0.00001, 0.63) 0,169	-15,40
MIRlet7c	-7,399	(0.00001, 0.33)0,231	-10,76	(0.00001, 0.29) 0,592	-8,297
MIR39	-1,644	(0.00001, 1.84)0,525	1,036	(0.00001, 5.26) 0,095	-2,236
MIR100	-3,871	(0.00001, 0.66)0,417	-3,834	(0.00001, 0.79) 0,218	1,153

Reference: Calderón J.F-Regulation of Connexins Expression Levels by MicroRNAs-Front Physiol.2016; PMCID:PMC5122916 Grant# TSG-2013-333.Research Fund of Istanbul Medeniyet University.

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E-P02.11

Whole exome sequencing identifies novel *TRIOBP* mutations as a cause of a distinct audiological manifestation

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Implementation whole exome sequencing (WES) has provided unique opportunity for a wide screening of mutations in genetically heterogeneous diseases including nonsyndromic hearing impairment (HI). Based on WES analysis, we identified two novel *TRIOBP* mutations (p.Gln268Leufs*610 and p.Gly1672*) causative of nonsyndromic, peri- to postlingual, moderate-to-severe hearing loss in three siblings from a Polish family. Typically, *TRIOBP* mutations lead to prelingual, severe-to-profound HI, thus the onset and degree of HI in our patients represent a distinct phenotypic manifestation caused by *TRIOBP* mutations. *TRIOBP* in the inner ear is responsible for proper structure and function of stereocilia and is necessary for sound transduction. The presented mutations disrupt *TRIOBP*-4 and *TRIOBP*-5 isoforms, forming the rootlets of stereocilia. Although *TRIOBP* mutations are not a frequent cause of HI, this gene should be included in multi-gene diagnostic panels, especially in patients with a postnatal hearing loss. A delayed onset of HI due to *TRIOBP* mutations creates a potential therapeutic window for future targeted therapies.

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E-P02.12

Efforts to decipher novel genes in 25 Iranian families presenting hereditary hearing loss using whole exome sequencing

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Hearing loss (HL) is the most common communication disorder affecting about 1/1000 births worldwide. Hereditary Hearing Loss is the second cause of disability in Iran. Recently, with using a custom capture panel (Otoscope) which include 116 known HL genes in a cohort of 302 *GJB2* mutation-negative deaf probands, we were able to perform the genetic diagnosis for 67% of families. The families, which failed to identify plausible disease-causing variants using targeted NGS, are a valuable cohort for novel deafness-gene discovery. The aim of this study is to determine the genetic cause of the remaining families by Whole Exome Sequencing (WES). So far, twenty-five families were subjected to WES. Whole exome sequencing was performed on proband of each families using Agilent SureSelect Human All Exon kit, sequenced on the Illumina Hiseq 2000. All data has been processed, annotated and analyzed with BWA, GATK, Annovar and other databases. Candidate mutations have been verified by cosegregation with the phenotype in all of the family members. Until now by analyzing the results of 13 families, strong candidate variants have been detected in five families. Further studies for confirmation of the pathogenicity of these variations and data analysis of remaining families are under investigation.

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E-P02.13

Further assessment of genes with altered expression level in keratoconus corneal tissues

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Introduction: We have demonstrated by an RNA-Seq approach an extensive disruption of collagen synthesis and maturation pathways, as well as dysregulation of the core elements of the TGF- β , Hippo, and Wnt signaling pathways influencing corneal organization in keratoconus (KTCN) human corneas (Kabza et al., Eur J Hum Genet. 2017 doi:10.1038/ejhg.2017.4.). Here, we focused on the revealed differentially expressed genes to verify down- and upregulated genes in KTCN corneas. Materials and methods: RNA samples extracted from corneal tissues of six KTCN and six non-KTCN non-related Polish individuals were used in this study. After reverse transcription, Real Time experiments using the RealTime ready Custom Panels (Roche) containing qPCR assays for 45 genes of interest normalized to reference genes, including *GAPDH*, *IPO8*, and *ACTB*, were performed in triplicate for every gene. Results and conclusions: We observed a significant down-regulation of *COL5A2*, *TGFB3*, *CTGF*, *TEAD2*, *TEAD4*, *ZNF469*, *SMAD7*, and *SPARC* genes and upregulation of *COL21A*, *SKP1* and *TGFB1* expressions in KTCN corneas, which was consistent with our previous RNA-Seq data. The specific profile of gene expression was shared by all KTCN individuals. Some expression profile similarities between an individual with corneal scar (after mechanical damage) and KTCN patients were observed. These results open discussion about genetic profiling in ocular diseases and mechanical damages in KTCN etiology. This work was supported by National Science Centre in Poland, Grant 2012/05/E/NZ5/02127 (to MG)

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E-P02.14

Targeted NGS identified a novel variant in LRP5 causing familial exudative vitreoretinopathy in an Iranian consanguineous pedigree

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Familial exudative vitreoretinopathy (FEVR) is characterized by incomplete development of the retinal

vasculature. It has variable expressivity even within families. Defective retinal angiogenesis, peripheral avascularity, and retinal ischemia can cause hyperpermeable blood vessels, vitreoretinal traction, retinal folds, and retinal detachments. FEVR is inherited in an autosomal dominant/recessive (AD/AR) manner. In this study a consanguineous family with two affected sibs of two and four years old were investigated. They had similar symptoms of aphakia, epiretinal membrane, pigmented retinal lesions, and unilateral funnel shaped folded retina. Target region capturing of 175 genes related to inherited eye diseases was performed on the younger sib's sample followed by next generation sequencing (NGS). The detected variant(s) were investigated by PCR-Sanger sequencing for validation/segregation analysis. A novel homozygous missense variant in *LRP5* gene was detected in the proband. It was also detected in homozygous state in her sister by Sanger sequencing. Segregation analysis was consistent with AR pattern of inheritance. This variant was absent in population (1000 G, ExAC, dbSNP) and disease-specific (ClinVar, OMIM, HGMD) databases, predicted to be disease-causing by multiple in-silico predictive tools (Mutation Taster, SIFT, PolyPhen, CADD) and the substituted nucleotide was evolutionarily well-conserved (ConSurf, phyloP, phastCons). Based on ACMG guidelines for interpretation of sequence variants, it is classified as a variant with uncertain significance; efforts to resolve this classification as possibly pathogenic is undertaken. Although mutations in *LRP5* are the most common cause of AR-FEVR, applying NGS in less investigated populations can still detect novel variants, even in well-studied genes.

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E-P02.15

Progress in the genetic diagnosis of inherited retinal dystrophies

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Inherited retinal dystrophies (IRD) encompass a group of disorders characterized by progressive loss of

photoreceptors resulting in legal blindness. The genetic diagnosis by traditional methods is impossible due to high genetic heterogeneity and clinical variability of the IRD. Therefore, the aim of this study is the molecular diagnosis by next generation sequencing of patients with IRD. We designed a panel including 117 genes and 5 deep intronic mutations described for this group of disorders. We used SureSelect^{QXT} (*Agilent*) protocol for the library construction and MiSeq (*Illumina*) platform for samples sequencing. Data were analysed by SureCall (*Agilent*) and wANNOVAR (*WGLab*) softwares. We used 8 patients with mutations of different nature previously detected in 8 genes responsible of IRD. The designed panel had a size of 490kbp. The 67% of total reads were aligned in our interest regions. The average depth was 228x. The 98% of bases had a depth > 50x. We detected the 100% of control mutations, consisting in: one splicing mutation, 2 missenses, 5 frameshifts and one 5 exon deletion; localized in the genes: *NR2E3*, *ABCA4*, *RPGM*, *RHO*, *PRPF8*, *CHM*, *PRPH2* and *USH2A*. The results of the control patients sequencing (coverage, reads depth and control mutations detection) validated this analysis as strategy for the molecular diagnosis of IRD. Once the panel was validated, we started to sequence a cohort of 100 patients clinically classified as IRD but without molecular diagnosis. Our results are promising as the detection rate of the panel is higher than 50% of all the patients analysed.

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E-P02.17 **Down-regulation of Pluripotency Markers, SOX2 and OCT4, predicts a new avenue for the involvement of LSCs in pterygium development**

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Pterygium is a benign growth of fibrovascular tissue on the cornea. It is a common ocular surface disease characterized by the abnormal epithelial proliferation, matrix remodeling, vascularization and the migration of the lesion. The etiology of pterygium is elusive but recent studies have focused on LSCs damage. The aim of this study was to examine the mRNA expression levels of *SOX2* and *OCT4* genes in pterygium patients. Using real time PCR, the expression of *SOX2* and *OCT4* were analyzed in primary

pterygium and their normal conjunctiva tissues. The correlation between *SOX2* mRNA expression with *OCT4* mRNA expression, as well as correlation between clinicopathological indices with both gene expression levels were assessed, statistically. The relative mRNA expression levels of *SOX2* and *OCT4* genes in primary pterygium tissues revealed significant decreased compared with normal conjunctiva tissues ($p = 0.04$ and $p = 0.05$ respectively). The correlation between *OCT4* gene expression and clinicopathological indices were significant in laterality ($p = 0.006$), grade T2 ($p = 0.007$) and recurrence ($p = 0.014$) indices. Finally, the statistical analysis showed significant correlation between expression of *SOX2* and expression of *OCT4* ($p = 0.004$). The outcomes of the present data highlighted the down-regulation of pluripotency markers, *SOX2* and *OCT4* genes, in the pterygium. It is speculated that these results may be predicted a new avenue for the involvement of LSCs in pterygium.

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E-P02.18

Identification of *POUBF4* gene deletion in two Mexican families with hearing impairment through MLPA

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Sensorineural hearing loss (SNHL) is a disease clinically and genetically heterogeneous. It is considered that 200 to 250 genes are involved in SNHL. Recently, more than 80 genes, around 1000 mutations, and 140 loci have been associated with SNHL (<http://hereditaryhearingloss.org/>). Current technologies play an important role in the diagnosis of different pathologies; MLPA is a tool in the characterization of the genetic etiology in SNHL. In this study, we describe the presence of *POUBF4* gene deletion in two families with SNHL from a sample of 23 non-related cases. One family also presented *PSP* and *ZMYM2* gene deletion. Analysis was performed through MLPA and PCR in all families and in 100 normal controls. Parents and non-affected members of the families showed absence of the molecular defect, all of them with normal audition. In conclusion, we describe two families with SNHL due to

POUBF4 gene deletion; this enriches the mutational gene spectrum in Mexican population in patients with SNHL.

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E-P02.19

MTHFR (677 C > T) gene polymorphism increase the risk of proliferative retinopathy in type 2 diabetes

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Introduction: Diabetic retinopathy (DR) (proliferative or non-proliferative) is progressive microangiopathy of the retina due to prolonged irregular blood glucose which eventually lead to blindness. Proliferative DR is more advanced type of DR and characterised with scar tissue and pathologic neovascularization, which are weak and bleed easily. Here, we aimed to asses whether thrombosis related gene polymorphisms [plasminogen activator inhibitor-1(*PAI-1*) and Methylene tetrahydrofolate reductase (*MTHFR*)] are associated to proliferative retinopathy.

Methods: A case-control study was conducted in patients with T2DM with proliferative retinopathy (n = 33), non-proliferative retinopathy (n = 33) and non diabetic controls (n = 33). Genomic DNA was extracted from whole blood and genotyped by Real Time PCR method and compared using one sided Fisher's exact test.

Result: The expected/observed genotype frequencies were all in equilibrium with Hardy-Weinberg. *MTHFR* (C677T)(rs1801133) polymorphisms were significantly different between proliferative and non-proliferative DR (p = 0,012), that having at least one T allele would 3.6 times increase the risk of having proliferative retinopathy in diabetic patients [OR = 3,619 (95% CI: 1,290–10,150)]. *PAI-1* 4 G/5 G gene polymorphism did not reveal any significant association.

Conclusion: We report that, genetic predisposition to increased blood clotting might increases the risk of proliferative retinopathy in diabetic patients.

Reference. Luo S et al, 2016. PMID: 27517946 **Keywords:** proliferative diabetic retinopathy, diabetic retinopathy, *MTHFR*, 677 C > T, gene polymorphism

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E-P02.20

Two different novel EYS mutations cause retinitis pigmentosa in a single Bedouin kindred

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Eleven individuals of two unrelated consanguineous Bedouin families presented with severe early-onset retinitis pigmentosa (RP). All affected individuals exhibited severe disease with an onset in the second or third decade of life and severely reduced and delayed ERG responses. Combining 750k SNP array for all family members with whole exome sequencing of two affected family members, we identified in this kindred two different mutations in *EYS* (RP25): a deletion mutation encompassing 10 of the 43 exons and a p.W1817* nonsense mutation. Segregation analysis of both mutations demonstrated that all affected individuals were either homozygous for either one of the mutations, or compound heterozygous for both mutations. Both mutations are predicted to cause loss of function of the encoded protein and were not present in 200 ethnically-matched controls. Thus, we demonstrate pseudo-dominant heredity of RP in a consanguineous Bedouin clan caused by homozygosity and compound heterozygosity for two different *EYS* mutations: a nonsense mutation and a micro-deletion. Our findings of two different mutations in the same gene in a single inbred kindred highlight the limitations of homozygosity mapping in disease-gene identification in inbred cohorts. Identification of the novel RP-causing mutations will facilitate early diagnosis, screening,

and genetic counseling in the Bedouin population. Funding: the study was supported by an Israeli ministry of health research grant and Foundation Fighting Blidness USA (BR-GE-0214-0639).

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E-P02.21

Compound heterozygous variants in *IFT140* as a cause of non-syndromic Retinitis Pigmentosa

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Introduction: We present a 22-year-old female patient who attended her opticians as she suffered from frequent headaches. It was found that her visual fields were restricted, and further investigations revealed the diagnosis of 'Retinitis Pigmentosa' (RP). She was referred to the Genetics clinic where 2 heterozygous *IFT140* variants were identified. *IFT140* is a ciliary transporter gene. Recessive variants have previously been reported in association with severe syndromic ciliopathy such as Mainzer-Saldino or Jeune syndrome, which is associated with RP, skeletal dysplasias and renal abnormalities. Materials and Methods: A total of 176 genes associated with retinal dystrophy were targeted using Retinal dystrophy v3 Agilent SureSelect Custom Design and sequenced on the HiSeq2500 (Illumina) system according to manufacturer's protocols. Sequence data was mapped with GenomeAnalysis ToolKitLite-v2.0.39 (GATK). Results: Compound heterozygous variants in *IFT140* NM_014714.3: c.4182 G>C p.(Thr1394Thr) and c.212 C>T p.(Pro71Leu) were identified, and were confirmed by Sanger sequencing. *IFT140* c.4182 G>C p.(Thr1394Thr) is a synonymous change but *in silico* prediction tools indicates that this change may reduce the splice donor site of intron 30. *IFT140* c.212 C>T p.(Pro71Leu) has previously been reported in the compound heterozygous state in a patient with non-syndromic RP. Conclusion: This report adds to the emerging literature of *IFT140* variants causing a non-syndromic RP and highlights to clinicians the need for confirming diagnosis through genetic testing to clarify recurrence risks. Further case reports with detailed phenotypic information are required to eventually provide genotype-phenotype correlation in *IFT140* gene, so we can identify variants only

causing non-syndromic RP rather than a syndromic ciliopathy.

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E-P02.22

A putative splice site mutation in *GUCA1B* co-segregates with autosomal recessive Rod-Cone Dystrophy

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Inherited retinal diseases (IRDs) represent a major cause of blindness in adults with an estimated prevalence of 1 in 2,000 individuals worldwide. The spectrum of these disorders is clinically and genetically heterogeneous. Genetic studies have highlighted more than 200 mutant genes involved in about 60% of the cases. A woman with sporadic rod-cone dystrophy (RCD), from a consanguineous family, was clinically investigated at the Quinze-Vingts hospital. Targeted next generation sequencing followed by whole exome sequencing was performed on the index patient and five unaffected family members. Stringent filtering keeping only variants in exonic and putative splice sites with a maximal allele frequency of ≤ 0.005 was applied. A variant in *GUCA1B* (c.357 + 6 T>C, p.?) was identified in the affected patient at homozygous state, which co-segregated with the phenotype in the family. Although the variant described herein does not directly affect splice acceptor or donor sites, it is predicted to influence splicing. Minigene approaches are underway to confirm these findings. *GUCA1B* encodes one of three guanylate cyclase-activating proteins, which is expressed in rods and cones. These proteins play a key role in the phototransduction cascade. Although *GUCA1A* mutations are well known to cause autosomal dominant IRDs, the implication of *GUCA1B* mutations in IRDs is controversial. Here we report a homozygous variant in *GUCA1B* in a sporadic RCD case most likely influencing splicing. Further studies are needed to confirm this hypothesis. These findings suggest that *GUCA1B* should be investigated in genetic screening of

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E-P02.23

The importance of an early genetic diagnosis in Usher syndrome

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Introduction: Usher syndrome (USH), the most prevalent cause of hereditary deafness-blindness, is an autosomal recessive and genetically heterogeneous disorder. The syndrome has three distinct clinical subtypes (USH1-3), which can be distinguished by their severity and age of onset. The use of next generation sequencing techniques has improved considerably the time and cost-effectiveness of the molecular diagnosis of USH. The aim of this study was to develop a custom panel of genes to early diagnose Usher syndrome patients in order to better plan auditory-visual rehabilitation strategies. Materials and Methods: Ten patients with clinical evidence of sensorineural hearing loss were assed and tested for germline mutations at Sant Pau Hospital. We have designed a panel including 22 genes related to nonsyndromic and syndromic hearing loss. Libraries were prepared using the TruSeq Custom Amplicon (Illumina) and sequenced on an Illumina MiSeq sequencer. All pathogenic variants were validated by Sanger sequencing and its inheritance was evaluated within each single family. Results: We have obtained high quality sequences of all the regions included in the panel (>99%) with coverages over 70×. Three patients were diagnosed as Usher type-1, one as type-2 and one as type-3 due to mutations in the *MYO7A* (OMIM 276900), *USH2A* (OMIM 276901) and *CLRN1* (USH3A, OMIM 276602) genes respectively. Conclusions: Studying genetics in sensorineural hearing loss patients will allow us to uncover causes which are presently unknown improving therapy planning. Usher patients can benefit from prevention, diagnosis, prognostic, treatment and genetic counselling.

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E-P02.24

A new case of Waardenburg syndrome associated with ocular albinism

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Waardenburg syndrome (WS) is characterized by sensorineural deafness and pigmentation abnormalities. WS can be caused by heterozygous alterations of *MITF*. Two unrelated families with an association of WS and ocular albinism were previously reported, suggesting a digenic inheritance in this phenotype. In these families a frameshift deletion of *MITF* was identified, co-segregating with the p.(arg402Gln) variant of *TYR* in the first family and p.(Pro513Arg) mutation of *TYRP1* in the second one. The contribution of p.(arg402Gln) in albinism is controversial in the literature, but it is suggested that this variant can be responsible for a mild form of oculo-cutaneous albinism, depending on the variant present in trans. Here we describe a 3 years old girl with ocular albinism, global hypopigmentation, congenital deafness and iris heterochromia. We performed next generation sequencing of a panel containing all known albinism genes and WS genes. We identified a heterozygous intragenic deletion in *MITF* and the homozygous p.(arg402Gln) variant in *TYR*. No other mutations were found. In this observation we support the hypothesis of an additive effect of *MITF* and *TYR* variants leading to simultaneous WS and albinism.

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E-P02.25

Waardenburg syndrome in the Sakha Republic (Eastern Siberia, Russia): mutation analyses of genes *PAX3*, *MITF*, *SOX10* and *SNAI2*

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The Waardenburg syndrome (WS) is a rare genetic disorder with autosomal dominant inheritance. WS leads to hearing loss and impairment of pigmentation of the skin, hair and eyes. The 393 patients from 364 families with congenital hearing impairment were clinically examined in the Sakha Republic (Eastern Siberia, Russia) and three affected subjects from two unrelated families with WS phenotypes were found among this cohort of patients. Thus, the frequency of WS in patients with congenital hearing impairment in the Sakha Republic is estimated as 0.76% (3/393). We performed sequencing of coding regions of genes *PAX3*, *MITF*, *SOX10* and *SNAI2* in affected members of these families. Two heterozygous likely nonpathogenic synonymous substitutions: p.Asn268Asn (*PAX3*) and p.His309His (*SOX10*), were found in two members of one family (father and his daughter) with the WS type I phenotype. Heterozygous transition c.775 C>T in exon 8 of gene *MITF* leading to a premature stop-codon (p.Arg259*) which is known to be associated with WS was detected in patient with the WS type II phenotype from another family. This patient is characterized by congenital unilateral hearing loss and heterochromia of irises (right eye is dark-brown, left is brilliant blue) and absence of telecanthus and depigmentation of skin/hair. Our study confirms involvement of mutations in the *MITF* gene in etiology of the WS type II. The study was supported by RFBR (16-34-00234mol_a, 16-34-00564mol_a), project №556 FASO_Russia, №6.1766.2017/ITI and NOFMU.

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E-P03

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

E-P03.01

Frequency of the 8 bp deletion in exon 3 of the CYP21A2 gene in Macedonian patients with classical 21-hydroxylase deficiency

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Introduction: 21-hydroxylase deficiency is an autosomal recessive endocrine disorder due to mutations in the CYP21A2 gene. Severe enzyme deficiency can present as a classical salt wasting (SW) and simple virilizing form (SV). The 8 bp deletion in exon 3 is a mutation transferred from the pseudogene which shifts the reading frame, thus producing inactive enzyme. This mutation is associated with SW phenotype. Materials and Methods: Fifty DNA samples from Macedonian patients with clinical and laboratory signs of severe form of 21-hydroxylase deficiency, 22 SW and 28 SV, were collected and subjected to polymerase chain reaction for the detection of presence of GAGACTAC sequence at the position 707–714 in exon 3 of the CYP21A2 gene. The patients were evaluated at the Department of Endocrinology and Genetics, University Pediatric Clinic, Skopje, Republic of Macedonia. Results: We detected presence of 8 bp deletion on the six (6/100) of the analyzed alleles (6%), of which 4/44 (9.1%) were in SW patients, and 2/56 (3.6%) in SV form. One of the SW patients, 4.5% (1/22), was a homozygous (8 bpdel/8bpdel), and two 9.1% (2/22) were compound heterozygote with genotypes 8bpdel/Q318X, and 8bpdel/R356W. Among the SV patients, one was compound heterozygote (3.6%) with genotype 8bpdel/V281L, and the other one (3.6%) was heterozygote for 8bp deletion with no detected mutation on the second allele. Conclusions: The frequency of 8 bp deletion in Macedonian patients with classical 21-hydroxylase deficiency, that is comparable to the most of the other European countries, supports its role in classical phenotype of the disease. 1 **V. Anastasovska:** None. **M. Kocova:** None.

E-P03.04**Antenatal Bartter syndrome type 1 with a novel homozygous *SLC12A1* mutation**

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Three affected children of single Bedouin kindred of the Negev region of southern Israel presented with premature birth in 28–29 week of pregnancy, accompanied by an increase in the amount of amniotic fluid, nephrocalcinosis, hydronephrosis, vesicoureteral reflux, disorders of fluid electrolyte and acid-base balance, hypercalcemia, lack of expected normal physiological development in childhood. Genome-wide linkage analysis followed by fine mapping identified a 3.3 Mb haplotype on chromosome 15, which was shared by and unique to the affected individuals in the kindred. Homozygosity common to all affected individuals was not found in any of the genomic loci of genes previously shown to be associated with Bartter syndrome (OMIM #601678), except *SLC12A1*, at 15q21.1. Within the 3.3 Mb locus, whole exome sequencing for an affected individual identified homozygous mutation in exon 22 of *SLC12A1*. Sequencing of the region of interest in this exon identified a novel missense mutation (c.2759 G > A), resulting in substitution of Glycine by Glutamic Acid at position 920 of NKCC2 (butyryl-CoA-sensitve Na-K-2Cl cotransporter)-protein, that is found specifically in the kidney, where it serves to extract sodium, potassium, and chloride from the urine so that they can be reabsorbed into the blood. Detected mutation segregated within the kindred as expected in recessive heredity.

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E-P03.06**Familial hypomagnesemia with hypercalciuria and nephrocalcinosis: New cases and identification of a novel *CLDN16* mutation**

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Introduction: Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is a rare autosomal recessive tubular disorder caused by mutations in *CLDN16* or *CLDN19*. These genes encode tight-junction proteins claudin-16 and claudin-19, respectively, which are involved in paracellular calcium and magnesium reabsorption in the kidney. FHHNC is characterized by excessive renal magnesium and calcium excretion in urine. Early in life, patients develop nephrocalcinosis and chronic kidney failure. Patients with mutations in *CLDN19* also present severe ocular abnormalities. Here, we describe the clinical manifestations and genotype of eight new cases. Materials and Methods: The medical records of two girls and a boy and five adults, two males and three females, diagnosed with FHHNC were examined. The *CLDN16* and *CLDN19* genes of patients and relatives were analysed by DNA sequencing. Bioinformatics tools were used to predict the consequences of mutations. Results: Three *CLDN16* mutations, c.571 G > A (p.G191R), c.485 G > T (p.G162V) and c.165_166delG-GinsC, and two missense *CLDN19* mutations, c.57 G > A (p.G20D) and c.83 C > T (p.P28L), all previously described, were detected. In addition, a novel *CLDN16* mutation, c.602 G > A (p.G201E), was identified. Bioinformatics analysis suggests that this presumed missense mutation also creates a new donor splice site and inactivates an exonic splicing enhancer. Conclusions: Genetic analysis confirmed the clinical diagnosis. Results obtained with adult patients suggest that FHHNC may be under-diagnosed. We suggest that the new mutation could alter splicing of the *CLDN16* pre-mRNA. This work was supported by grant PI14/00760, co-financed by Instituto de Salud Carlos III (Spain) and the European Regional Development Fund “A way to build Europe”.

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E-P03.07**Mutational analysis of *CLCN5* and *OCRL* in patients with Dent disease**

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Introduction: Dent disease is an X-linked renal tubulopathy characterized by low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis and progressive renal failure. Two-thirds of the cases are associated with inactivating mutations in the *CLCN5* gene and a few present mutations in *OCRL1*. Our objective was to describe the clinical and genotypic features of new patients diagnosed with Dent disease. Materials and methods: Nine male patients with suspected Dent disease were studied. The age range at the time of diagnosis was 0.8–19 years. All patients presented tubular proteinuria, six presented hypercalciuria and six had nephrolithiasis or nephrocalcinosis. The *CLCN5* and *OCRL1* genes were analysed by PCR amplification of the coding exons and DNA sequencing. Results: Eight patients revealed *CLCN5* mutations and one an *OCRL1* mutation. A novel *CLCN5* mutation c.1641G>T (p. W547C) was identified. This presumed missense mutation, located in a mutation hotspot, also activates an exonic cryptic donor site and creates an exonic splicing silencer motif. In one patient PCR products could not be obtained using any of the *CLCN5* specific primers, indicating the presence of a *CLCN5* large deletion. The remaining mutations were three nonsense, two missense, and two small deletions that had been previously described. Conclusions: All the mutations identified predict important structural and functional changes in CIC-5 or OCRL proteins. We suggest that mutation c.1641G>T can result in alterations of *CLCN5* pre-mRNA splicing. This work was supported by grant PI14/00760, co-financed by *Instituto de Salud Carlos III* (Spain) and the European Regional Development Fund “A way to build Europe”.

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E-P03.08***POU1F1* and *PROP1* gene mutations in 4 cases of combined pituitary hormone deficiency**

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Introduction: Combined pituitary hormone deficiency (CPHD) is characterized by the impaired production of GH together with one or more other pituitary hormones. The most common recognized genetic defects associated with CPHD include mutations within *PROP1*, *POU1F1*, *HESX1*, *LHX3*, *LHX4*, *OTX2*, *GLI2*, and *SOX3*. The phenotype connected to *POU1F1* mutations is characterized by profound GH and PRL deficiencies, variable degrees of TSH deficiency, severe proportional short stature, atypical facies and feeding difficulties in infancy. Patients harboring mutations within *PROP1* gene present GH, PRL, TSH deficiencies in addition to variable defects in LH/FSH and ACTH secretion. Here, we present 3 cases with *POU1F1* mutation and 1 case with *PROP1* mutation, who were molecularly diagnosed in the Medical Genetics Department of Ege University.

Result: Three cases (1 female, 2 males) carrying *POU1F1* mutations all had short stature. One male case with a novel mutation, p.K216T also presented with micropenis in addition to short stature. The other mutations detected in *POU1F1* gene were S50A, R265W; S50A being novel. Fourth case with *PROP1* mutation also had short stature and micropenis. Molecular analysis revealed a frameshift p.L102CfsX8 mutation in the *PROP1* gene. Biochemical testing showed PRL and GH deficiencies in all cases. Two cases with *POU1F1* defects and a case with *PROP1* defect also had central hypothyroidism.

Conclusion: It's considered that in patients with growth retardation together with combined pituitary hormone deficiency *POU1F1* and *PROP1* gene mutations should be investigated. In this study two novel *POU1F1* mutations have been defined for the first time.

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E-P03.09

Can we trust genotype-phenotype correlations of IVS2 mutation?

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Introduction: Congenital adrenal hyperplasia (CAH) occurs mostly (%90) as a consequence of 21-hydroxylase deficiency. Although the frequency differs according to the region, nine mutations in the CYP21A2 gene (IVS2-13 A/C > G, V281L, Q318X, R356W, ClusterE6, P30L, I172N, 8 bp deletions, large deletion) are typically defined in these patients. Genotype-phenotype correlations of these mutations are fairly well known. IVS2 mutation accompanies classic CAH. Here we report two patients with homozygote IVS2 mutations together with their family, who have different clinics. Materials and methods: Pedigree charts were drawn for two patients, with classic and non-classic CAH. Addition to proband, siblings and parents were also analyzed. PCR/RFLP was performed for frequently identified nine mutations. Subsequently, the diagnosis was confirmed by sequencing. Results Homozygote IVS2 mutation is determined in the proband who has nonclassic CAH. The same mutation was found to be homozygous in her brother and mother whereas it was heterozygous in her father, all of them without any symptoms. The second patient with ambiguous genitalia had homozygote IVS2 mutation. Her father got heterozygote mutation, as expected. However, there was no clinical symptom in her mother although she had homozygote IVS2 mutation Conclusion: Homozygote IVS2 mutation has a well-known relation between phenotype and associated classic CAH. We described this mutation in patients with different clinical findings. When healthy members of these patients' families were investigated, phenotypic effects of this homozygote mutation were proved to be more complex. It should be noted that genotype-phenotype correlations of the CYP21A2 gene mutations could not always be valid.

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E-P03.10

A case of vitamin D dependent rickets type 1a with a novel mutation in the Turkish population

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Introduction: Vitamin D-dependent rickets type 1A (VDDR-1A)(Online Mendelian Inheritance in Man#264700) is autosomal recessively inherited,rare disorder due to mutations in CYP27B1 gene.Methods and Results: We report a boy referred to our clinic when he was 2 years old with Vitamin D dependent rickets type 1A presented with with inability to walk and bowed legs. He was born by spontaneous delivery to a healthy, non-consanguineous couple.His weight was 3250 gr at birth and given Vitamin D prophylaxis. At a physical examination, he was 76 cm tall (-3,13 SDS), weighed 10,3 kg (-1,9 SDS) and head circumference was 47 cm (-1,13 SDS). There was caput quadratum, genu varum and widened metaphyses of the wrists. His laboratory findings were; serum calcium (Ca = 8.1 mg/dl), phosphate levels (P = 3.37 mg/dl), serum alkaline phosphatase (ALP = 1383 U/L), magnesium (Mg = 2,12 mg/dl), parathyroid hormone (PTH = 787 pg/mL), serum 1,25(OH)₂ D₃ level was 10 pg/mL. Radiological findings were; cupping and fraying of the metaphyseal regions of ulna-radius and femur-tibia. The patient was treated with calcium carbonate and calcitriol. When patient was 6 years old, his calcitriol dosage was 15 ng/kg/day and he was 110 cm tall (-1,04 SDS), weighed 19 kg (-0,75 SDS). Consequently, CYP27B1 was analyzed by sequencing of the entire coding region (exons1-9) and a novel mutation NM_000785.3(CYP27B1): c.400_401insATT was described.Mutation taster predicts this variant as a prediction disease-causing mutation. Other members of the patient's family (mother,father and elderly sister) were heterozygous carriers for this novel mutation. Conclusions: The current study further expands the CYP27B1 mutation spectrum.

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E-P03.11**Unexpected results in an assay commonly used in cystic fibrosis diagnostics: the role of DNA sequencing of the CFTR gene in atypical electrophoretograms**

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Introduction: The Elucigene CF-EU2TM assay is being widely used for cystic fibrosis (CF) diagnostics, carrier testing and CF newborn screening (NBS). In some instances, interpretation of its results may be difficult since this assay could also be affected by intra-*CFTR* insertions and/or deletions within amplified target sequences or variants under proprietary primer annealing sites.

Methods: Manufacturer-recommended protocols for the Elucigene CF-EU2TM assay and the internal diagnostic database were used.

Results: During the last decade we observed 6 atypical findings: 4 in CF NBS, one in an infertile male and in CF carrier testing each. All 4 neonates were heterozygous for the F508del mutation, carrier testing detected heterozygous pathogenic variant 3849 + 10kbC > T, while in an infertile male no other variant was detected. Nonetheless, due to additional “suspicious” observations in assay electrophoretograms (e.g. absence of wild type peak in I507del, extra peaks in mixture B or normal height 3849 + 10kbC > T wild type peak) we performed targeted Sanger DNA sequencing of the corresponding exons/introns. We found a second *CFTR* variant in 3 cases (G509D, 2789 + 2insA, I132Cfs*26), including a non-CF-causing variant (F508C) and a variant of unknown significance (VUS) within carrier testing. Moreover, positive assay results in two infertile men were influenced by cross reactivity with another *CFTR* variant.

Conclusions: The utilised assay is highly reliable, but targeted DNA sequencing of the *CFTR* gene should be considered in instances when atypical electrophoretograms/positive results are being observed. Supported by 00064203, OPPK CZ.2.16/3.1.00/24022 and NF-CZ11-PDP-3-2014.

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E-P03.12**Targeted Next-Generation Sequencing identifies two novel *CFTR* gene mutations in Iranian patients with Cystic Fibrosis**

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Introduction: Cystic fibrosis is an autosomal recessive genetic disorder that has been associated with mutations in the *CFTR* gene which encodes a 1480-amino acids protein is called Cystic fibrosis transmembrane conductance regulator. CF affects often the lungs, but also the intestine, kidneys, liver, and pancreas. CF outbreak has been described as the highest among the Caucasians (1 in 2500) and two thousand cystic fibrosis-causing mutations have been described to date. The Targeted Next-Generation Sequencing (NGS) has been proved to be an effective strategy for the detection of mutations in CF. Materials and methods: The target region capture with Nimblegen chip in the gene of interest, followed by NGS. Results: The mutational analysis of the *CFTR* gene was performed in total 18 families with at least a patient suspicious to CF. Two novel mutations (c.1544_1547delATAG and c.175_176delAG) and four previously reported mutations (c.1163 C > T, c.1000 C > T, c.1520_1522delTCT, c.3107 C > T) were identified by NGS. Conclusions: Detection of two novel mutations among the eighteen families we studied, indicated that the profile of *CFTR* mutations in the Iranian patients are different from the mutations found in the other populations. Accordingly, the molecular analysis of more Iranian patients is necessary to find further novel mutations specific for Iranian population. In addition, the results of the assessment demonstrated that the targeted NGS is enable to identify *CFTR* mutations with high accuracy and speed. Thus, the technology can be used for the clinical diagnosis of the other Mendelian diseases.

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E-P03.13
***CFTR* mutation spectrum in Russia**

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Cystic fibrosis (CF) is the most common autosomal recessive disease among the Caucasian population. Over 2000 different mutations in the *CFTR* gene have been reported and the majority are extremely rare. The *CFTR* mutation detection rate varies by test method and ethnic background. Using literature data and Russian CF Patient Registry data the 40 most common mutations were selected. A sample of 386 CF patients examined: *CFTR* mutations identified on both chromosomes in 319 patients, on a single - in 67 patients. The allelic frequencies of 40 mutations were distributed as follows:: F508del (53,6%), E92K (6,5%), CFTRdele2,3 (21 kb) (5,3%), 1677delTA (3%), 3849 + 10kbC > T (2,8%), W1282X (2,3%), 2143delT (2,2%), N1303K (2,1%), 2184insA (1,8%), L138ins (1,8%), 3272-16T > A (1,3%), 394delTT (1,0%), G542X (1,0%), W1282R (0,9%), S466X (0,6%), R1066C (0,6%), R334W (0,5%), 2789 + 5 G > A (0,5%), 1898 + 1 G > A (0,4%), 4015delA (0,4%), S1196X (0,3%), S945L (0,3%), R347P (0,3%), 1367del5 (0,3%); 8 mutations (604insA, 621 + 1 G > T, 712-1G > T, R553X, S1159P, G85E, 3120 + 1 G > A, CFTRdup6b-10) met with a frequency 0,1%; 8 mutations (3821delT, I507del, 3944delGT, W1310X, 3849 G > A, G551D, 3272-26 A > G, 4022insT) did not meet at all. Total allelic frequency of 40 studied mutations in the sample of patients accounted 90,9%. Typical features of *CFTR* mutations distribution in Russian CF patients were lower frequency of mutations which are predominant worldwide, such as F508del, G542X, N1303K, G551D and other. On contrary, CFTRdele2,3, E92K, 2184insA, 2143delT, 1677delTA, L138ins mutations, which are quite rare in Western Europe were encountered more often in Russia. «Mild» mutations were more common in Russian population of CF patients compared to European countries.

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E-P03.14

The frequencies of CFTR M470V, intron 8 poly-T and pathogenic mutations in cystic fibrosis patients

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Cystic fibrosis (CF) is a chronic, multisystem, frequently lethal and autosomal recessive disorder caused by mutations in the *CFTR* gene which affects chloride transport. *CFTR*-related disease may arise because of multiple combining effects, such as modifier genes, epigenetic and environmental factors and mutations in genes that affects CF phenotypes. The IVS8-5T allele is associated with less effective use of the intron 8 splice acceptor site compared with the 7 T and 9 T alleles and causes frequent skipping of exon 9. The genomic DNA sequences of 100 patients with positive results for IRT (immunoreactive trypsinogen) or sweat tests were amplified by using the PARseq *CFTR* panel and sequenced using Ion Torrent S5 runs then analyzed with bioinformatic pipeline in routine diagnostic conditions and results are shown on the table. We obtained 100% coverage for all the target regions. NGS results were confirmed by the sanger sequencing method. Our results showed that M470V polymorphism and 5 T may affect phenotypic severity in CF. Systematic screening for *CFTR* mutations should contain the IVS8-5T allele for correct results. M470V polymorphisms may play a role in the development of CF-like disease and causes clinical variability. In addition, we concluded that *CFTR* sequencing assay by the NGS based test has increased the sensitivity and reliability of the assay than the traditional sanger sequencing. Table 1: NGS results CF patients

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CFTR NGS Results	n = 54 (100)	M470V (c.1408 A > G) Genotype distribution	n = 46 (100)	Poly T haplotype	n = 100
			MM	54% 7 T/7 T	62%
c.1521_1523delCTTCompound	1				
c.1712 T > C Heterozygote					
c.1521_1523delCTTCompound	7				
c.1408 A > G Heterozygote					
c.1521_1523delCTTHeterozygote	8				
c.1712 T > C Compound	4				
c.1408 A > G Heterozygote					
c.2991 G > C Heterozygote	3				
c.2991 G > C Compound	5				
c.1408 A > G Heterozygote					
c.224 G > A Heterozygote	4				
c.328 G > C Heterozygote	1				
c.3935 G > A Heterozygote	1				
Normal Genotype	20				
				Poly T allele frequency	
				5 T	7%
				7 T	78%
				9 T	15%

E-P03.15**The novel p.Cys1410* mutation causes severe neonatal CF in a Western Sub-Saharan African family**

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Despite continuous improvement of *CFTR* mutations detection kits in Caucasian population, diagnosis of Cystic Fibrosis (CF) remains poor in Western Sub-Saharan Africa (SSA), which is the area with the highest child mortality worldwide. Moreover, little is known about the mutations to search according to their geographical origin in SSA patients. This can make molecular diagnosis difficult, especially when phenotypic presentation cannot be explored (no sweat test for example), such as antenatal suspicion of CF due to fetal ultrasound digestive abnormalities. We report here the case of a consanguineous Senegalese couple. Their 1st child died at day one of life in a context of meconial peritonitis. Although fetopathological analysis revealed a digestive pattern compatible with CF, molecular analysis was not performed at this time. Fifteen years later, another pregnancy showed at the 2nd trimester the ultrasound triad associating hyperechogenic bowel, moderate loop dilation and non-visualization of the gallbladder in the fetus. The novel heterozygous c.4230 C > A, p.(Cys1410*) nonsense mutation was identified in both parents. This mutation should not elicit nonsense-mediated mRNA decay (NMD) and is predicted to delete the PDZ domain of the CFTR protein. The parents decided not to test their fetus through invasive procedure, and the diagnosis of CF was confirmed at birth (meconial ileus, typical phenotype, homozygous mutation). This case (i) reinforces the need to perform extensive genetic analyses when the ultrasound triad is observed, (ii) pinpoints that the diagnosis of CF should be considered in SSA patients, and (iii) shows the importance of clinico-biological discussion and genetic counseling.

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E-P03.16**Identification of causative gene(s) for Chronic Intestinal Pseudo-Obstruction by Next Generation Sequencing**

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Introduction: Developmental defects affecting the enteric nervous system (ENS), smooth muscles cells (SMCs) or interstitial cells of Cajal (ICC), result in chronic intestinal pseudo-obstruction (CIPO) syndromes. We have found heterozygous missense mutations of the *ACTG2* gene in a proportion of patients with either CIPO or MMIHS (microcolon, megacystic intestinal hypoperistalsis syndrome), as well as a *MYH11* variant in a patient showing MMIHS (unpublished), thus confirming genetic and clinical heterogeneity of the CIPO spectrum.

Methods: Whole Exome Sequencing (WES) approach has been applied to two sporadic cases with clinical history of severe constipation and recurrent sub-occlusions, and their parents. In addition, a three affected and one healthy children family and two affected sibs, all diagnosed with CIPO and with parents healthy and non-consanguineous, have also undergone WES analysis.

Results: To date, we have got results from two of the four families under study. No variant or mutant gene shared by affected members of these two families was identified. Segregating phenotypes must therefore be ascribed to different genetic causes. Overall, segregation and possible involvement of *AHNAK2*, *BMP8B*, *FAM86B2* and *LRRIQ1* genes are considered at the moment. Detailed results will be presented.

Discussion: CIPO includes heterogeneous disorders that are still very challenging in terms of diagnostic assessment, possible therapeutic interventions and genetic counselling. Finding genetic causes can be of great benefit to families and improve our understanding of the correct development of intestinal motility

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E-P03.17**Novel compound heterozygous mutations of growth hormone receptor gene in two Romanian siblings with Laron syndrome**

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Introduction. Many mutations in patients with growth hormone insensitivity syndrome from different world areas have so far been described.

Methods. Two Romanian siblings with Laron syndrome are presented, for which DNA analysis was performed by sequencing of exons 2 - 6 of the growth hormone receptor (GHR). Both patients had characteristic clinical features, including severe statural deficits and very low levels of insulin-like growth factor 1 (IGF1).

Results. DNA analysis revealed compound heterozygous mutations of the growth hormone receptor: Y86D (missense mutation) and c.440-1 G > A (splice mutation), the latter of which has not previously been reported.

Conclusion. This study reported two Romanian patients with Laron syndrome and a new compound heterozygous mutations was described.

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E-P03.18**Three generations of an Israeli family diagnosed with autosomal dominant activating mutation of the calcium sensing receptor (CaSR) gene**

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Introduction: Autosomal dominant hypocalcemia-1 (HYPOC1) HYPOC1HYPOC1 HYPOC1is a rare genetic disorder, characterized by low serum parathyroid hormone, mild-moderate hypocalcemia, hypercalcuria and a risk for nephrocalcinosis or kidney stones. Some patients

experience paresthesias, carpopedal spasm, seizures and basal ganglia calcifications. HYPOC1 is caused by heterozygous activating (gain of function) mutations in the CASR gene, encoding the calcium sensing receptor (CaSR). The activating mutations result in increased sensitivity of parathyroid and renal cells to calcium levels, leading to hypocalcemia being perceived as normal. Methods/patients: We describe a newborn, who presented with hypoparathyroidism, moderate hypocalcemia and hypercalcuria. Treatment with calcium and vitamin D supplementation was started. The infant's father and his paternal grandfather had similar laboratory findings and were also medically treated. Results: CaSR gene sequencing identified a missense activating mutation in exon 7 (c.2488 G > A - p.G830S). Following the diagnosis of HYPOC1, medical treatment was gradually reduced without any clinical manifestations. Conclusions: To the best of our knowledge, this report is the second molecular description of HYPOC1 in Israel. Since treatment with vitamin D supplementation to correct the hypocalcemia in patients with HYPOC may cause hypercalcuria, nephrocalcinosis and renal impairment, it is crucial to distinguish these patients from those with other forms of hypoparathyroidism.levels of PTH. Most of the affected members This report highlights the importance of molecular diagnosis of hereditary conditions, which may have implications for the medical therapy and follow-up. levels of PTH. Most of the affected members levels of PTH. Most of the affected members

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E-P03.19**HFE gene mutations analysis in hereditary hemochromatosis type1 and controls**

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Hereditary hemochromatosis type 1 (HH) is a common autosomal recessive disorder of iron metabolism. It is characterized by progressive iron overload and caused by mutation in the HFE gene on chromosome 6. The predominant feature of HH is excessive absorption of dietary iron and its deposition in parenchymal tissues and results in cirrhosis, diabetes, skin pigmentation, testicular failure, arthropathy and others. Prevalence is estimated at 1 in 200 to 1 in 2000. 32 HH patients (10 females and 22 males) and

106 healthy controls were screened for the Cys282Tyr, His63Asp and Ser65Cys mutation, using PCR-RFLP technique. All patients had the following parameters: iron studies including serum Fe, ferritin and transferrin saturation, serology for hepatitis B and C, liver function tests and abdominal echography. The mean age at genotype diagnosis was 54.1 years in males and 53.2 years in females. 27 from 32 (84.4%) HH patients were homozygous for Cys 282Tyr mutation. Four (12.5%) were compound heterozygous for Cys 282Tyr/His63Asp and one (3.1%) was for Cys282Tyr/Ser65Cys. Seven (6.6%) of our 106 controls were heterozygous for Cys282Tyr and two (1.8%) were heterozygous for His63Asp. Hereditary hemochromatosis type 1 is an underdiagnosed disorder. The most frequent form is associated with homozygosity of the Cys282Tyr mutation. Because of the late manifestation of HH, it should be considered in any patient with dysfunction of parenchymal tissues.

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E-P03.21

Pathogenic and possibly pathogenic genetic alterations of the *GH1* and *GHRHR* genes detected in a cohort of IGHD children in Sri Lanka

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Introduction: Several genetic studies have proved that alterations in *GH1* and *GHRHR* genes lead to isolated growth hormone deficiency (IGHD). The present study was designed to screen pathogenic genetic alterations in a cohort of IGHD children in Sri Lanka. Materials and Methods: Fifty five unrelated IGHD children negative for codon 72 (*GHRHR*) mutation were screened for gross *GH1* gene deletion by PCR-RFLP technique. The coding, intronic and promoter regions of the *GH1* gene (N = 53) were screened by direct sequencing in children who were negative for *GH1* deletion. In a subset (N = 40), coding, flanking intronic and promoter regions of the *GHRHR* gene were screened by SSCP/sequencing. Results: Eighteen children were found to carry pathogenic and possibly pathogenic genetic alterations. Gross *GH1* gene deletions (6.7 kb and

7.0 kb) were observed in two children. Three pathogenic variants (CM012951, rs863223309 and rs11568828) and three possibly pathogenic variants (rs140576665, rs779374598 and rs41295043) of the *GH1* gene and its promoter were observed in thirteen children. Three possibly pathogenic variants including one novel variant (Novel: c.211 G > T, rs201267804 and rs758281879) of *GHRHR* gene were observed in three children. The novel variant leads to an amino acid transition from hydrophobic Glycine to moderate Cysteine at codon 71. The pathogenic effect of the variant was predicted through in-silico analysis tools available online. Conclusion: Five pathogenic and three possibly pathogenic genetic alterations of *GH1* gene and its promoter and three possibly pathogenic variants of *GHRHR* gene were detected in this cohort. Supported by National Science Foundation, Sri Lanka (RG/2011/BT/03)

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E-P03.22

More secure milk consumption with genetic test - "School Milk" in Turkey

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Introduction: "School Milk" project is a nationwide governmental dietary cow milk consumption project in Turkey for primary school students to prevent them for calcium related bone diseases. However, lactose intolerance is major restriction for them due to intolerable destructing symptoms. We aimed to determine the underlying genetic causes of lactose intolerance since there is no definite diagnostic method. By developing and applying genetic test to the students, we also aimed to give special preliminary genetic counseling for the students before School Milk consumption.

Materials/Methods: A questionnaire on milk consumption and dairy products was prepared to identify lactose intolerance of the subjects. Oral epithelial samples were taken from 40 people, selected among 200, according to the questionnaire conducted. rs4988235 polymorphism of *LACTASE* gene was determined as the target after literature review. Individuals DNA isolated and polymorphisms were analyzed performing Real Time-PCR.

Results: The associations and genotype/phenotype correlations were analyzed using Pearson's chi-square test on IBM SPSS version 22 statistical program. There were significant relations between c.-13910C>T genotype and the subjects having symptoms of bloating ($p = 0.002$), nausea ($p = 0.001$) and hyperflatulence ($p < 0.001$).

Conclusion: We for the first time reported c.-13910C>T polymorphisms to be associated with symptoms of lactose intolerance, up to the literature. It would be meaningful to apply "Lactose Intolerance Genotype Test" to prevent clinical symptoms of lactose intolerance with inadvertent affects as fear and psychological trauma among children who consume the distributed School Milk and might have the clinical symptoms.

Reference: Bodlaj G-2006-Genotyping of the lactase-...

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E-P03.23

MAMLD1 deletions in three patients with proximal hypospadias

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Hypospadias is a congenital malformation that has a prevalence of 4–43:10.000. Distal or (sub)glandular hypospadias is far more common than proximal hypospadias. In most cases isolated distal hypospadias shows multifactorial inheritance, while the more proximal anomalies are part of the spectrum of Disorders of Sex Development (DSD) and can have a variety of genetic defects. *MAMLD1* is one of the genes associated with hypospadias, although functional studies in mouse KO models do not support this as the male knockout mice do not show hypospadias and have normal fertility^{1,2,3}. Patients with a contiguous syndrome involving the *MTM1* gene and *MAMLD1* have been described as well as patients with deletions encompassing *IDS* and *MAMLD1* not showing hypospadias. We present the findings in two sibs with proximal hypospadias and a very small deletion of *MAMLD1* confirmed with MAQ assay and a third patient with Hunter syndrome and hypospadias carrying a deletion encompassing the *IDS* gene, which extends to the first exon of transcript 1 of *MAMLD1*. ¹Ogata T, Sano S, Nagata E, Kato F, Fukami M. *MAMLD1* and 46,XY disorders of sex development. Semin Reprod Med. 2012;30:410–416 ²Camats N, Fernández-

Cancio M, Audi L, Mullis PE, Moreno F et al. Human *MAMLD1* gene variations seem not sufficient to explain a 46,XY DSD phenotype. PLoS ONE 2015; 10 (11):1–20. ³Miyado M, Nakamura M, Miyado K, Morohashi K-I, Sano S et al., Mamld1 deficiency significantly reduces mRNA expression levels of multiple genes expressed in mouse fetal Leydig cells, but permits normal genital and reproductive development. Endocri 2012;153(12):6033–6040

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E-P03.24

A recurrent mutation in *IER3IP1* causing microcephaly with simplified gyral pattern, epilepsy and permanent neonatal diabetes syndrome (MEDS)

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Microcephaly with simplified gyration, epilepsy and permanent neonatal diabetes syndrome (MEDS) is a recently described autosomal recessive inherited syndrome caused by mutations in the *IER3IP1* gene. We report the case of an infant male who presented at five weeks of age with lethargy and severe ketoacidosis. He required ICU admission with difficult management of persistent hyperglycaemia requiring high doses of insulin with poor response. Clinical examination revealed microcephaly without other dysmorphic features. MRI showed microcephaly with simplified gyration. The particular association of neonatal diabetes with the characteristic microcephalic pattern prompted the diagnosis of microcephaly with simplified gyral pattern, epilepsy and permanent neonatal diabetes syndrome (MEDS). The diagnosis was confirmed by the detection of a homozygous c.233 T>C mutation in the *IER3IP1* gene. This mutation has been previously described in three other MEDS cases of different geographic origins. Given that only seven patients with MEDS have been reported so far, this recurrent mutation may account for a notable proportion of MEDS cases (75%) although no clear founder effect can be assumed. The description of this patient should help to better delineate clinical manifestations of MEDS allowing earlier accurate genetic counseling.

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E-P03.25

Molecular diagnostics of MEN1 and MEN2 syndrome in Croatia

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Introduction: Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant syndrome caused by mutations in *MEN1* gene. It is characterized by development of endocrine and non-endocrine tumors (parathyroid tumors, pituitary tumors, gastrinomas, insulinomas, cutaneous tumors, etc.). Multiple endocrine neoplasia type 2 (MEN2) is a hereditary syndrome caused by mutations in *RET* proto-oncogene. It is classified in three autosomal dominant subtypes (MEN2A, MEN2B and FMTC) that involve high risk for development of medullary thyroid carcinoma.

Materials and Methods: 11 samples of genomic DNA were analyzed for MEN1 and 19 samples for MEN2. Sequencing of *MEN1* included the whole coding region, while for *RET*, only exons 10, 11, 13, 14, 15 and 16 were analyzed. For identification of mutations in the coding region of these genes, Applied Biosystems 3130xl Genetic analyzer and BigDye® Terminator v3.1 Cycle Sequencing Kit were used.

Results: In 3 patients with MEN1, heterozygous mutations were found (p.Arg415X; p.Lys120del; p.Gln393X). In 5 patients with MEN2, heterozygous mutations were found (p.Met918Thr; p.Cys620Arg; p.Asn777Ser). Mutation p. Cys620Arg was found in three out of five members of the same family.

Conclusions: Molecular diagnostics of MEN2 is important because of the genotype-phenotype correlations that can be used for risk prediction of aggressive medullary thyroid carcinoma. Contrary to that, MEN1 syndrome shows no genotype-phenotype correlations, however molecular diagnostics of MEN1 is relevant for identification of at-risk family members.

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E-P03.26

A novel HNF1B mutation in a family with two MODY patients

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Introduction: MODY (maturity-onset diabetes of the young) is a form of monogenic diabetes characterized by hyperglycemia with childhood or early adult-onset. HNF1A and GCK mutations are responsible for %50 of MODY cases. More rarely HNF1B mutations cause a clinically distinctive type of MODY (Type 5) presenting with urogenital abnormalities. HNF1B gene encodes a transcription factor important in kidney and pancreas development. Case Presentation: A 34 year-old male patient was referred to our department because of diabetes mellitus who had complaints of polyuria and polydipsia for ten years. Despite insulin and oral antidiabetic treatments, his blood glucose levels remained over 200 mg/dl. His mother and aunt also had diabetes. He was overweight (BMI:28). Biochemical evaluation revealed hyperglycemia, hypertriglyceridemia, high HbA1C, normal insuline and C-Peptide levels. No urogenital abnormality was found in ultrasonographic evaluation. Mutations in seven genes (HNF1A, GCK, INS, HNF4A, ABCC8, KCNJ11 and HNF1B) which are common causes of MODY were screened via next-generation sequencing. We found a novel heterozygous variant c.1484 T>A (p.Met495Lys) in HNF1B gene. The mutation was also present in his mother having similar clinical and biochemical findings. In silico analyses of the variation indicated that it is a damaging mutation. Conclusion: We reported a novel heterozygous HNF1B mutation in two MODY patients with hypertriglyceridemia but without urogenital abnormalities. HNF1B mutations are also related with late onset Type II DM. Our patients are diagnosed at their third decade. Functional studies and more reports are required to understand the effect of the mutation and for genotype-phenotype correlations.

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E-P03.27**Mutation analysis of the CFTR gene in atypically mild cystic fibrosis patients**

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Cystic fibrosis (CF) presents with broad phenotypic variability, even in patients with identical mutations in the causative gene *CFTR* encoding for cystic fibrosis transmembrane conductance regulator. *CFTR* gene was studied in unrelated Russian CF patients with atypical form of disease. We performed the sequencing analysis to characterize mutations in *CFTR* (27 coding exons, splice-site junctions of the *CFTR*, 5'-UTR region including DNase hypersensitivity site located at a distance -20,9 kb from start codon). The overall efficiency of the CF allele detection after sequencing was 80%. The most frequent mutated allele, *delF508*, was identified in 25% of CF chromosomes. One patient had a large deletion from 1 intron to 3 exon (*CFTR21kbdel*). One novel nonsynonymous mutation was found *p.Lys1468Arg*. We identified 4 polymorphic variants with quite high frequency in group (*470Met/Val*, *4700T8/9*, *1393-61A/G*, *-8G/C*). To analyze the potential involvement of polymorphic variants in the pathogenesis of CF we used the web-tool "Human splicing finder". It was showed that the polymorphism 2562 T/G can induce the alternative splicing site of *CFTR*. We hypothesize that variations in CF severity can be associated with the combination of several polymorphic alleles of *CFTR*. This research was supported by a grant of the Charitable Foundation «Ostrova»

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E-P03.28**NPHS2 R229Q gene polymorphisms and steroid-resistant nephrotic syndrome**

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Background: Nephrotic syndrome (NS) is the most common glomerular pathological condition encountered in children. The role of NPHS2 R229Q gene polymorphism in various renal disorders has been investigated. The relationship of gene R229Q polymorphism (p.R229Q) of NPHS2 with adolescent- or adult-onset steroid-resistant NS in European and South American populations has already been reported. The reason for selecting this single nucleotide polymorphism of NPHS2 gene is that it may influence susceptibility or clinical course of the disease. We undertook this research to study the genetic polymorphisms of NPHS2 R229Q in romanian children with NS, as well as its association with patient's clinical response to steroid therapy.

Material and method: This study was conducted on 67 nephrotic syndrome (INS) patients and 60 controls (without renal diseases and without proteinuria). The study groups consisted of 67% cases with steroid-sensitive nephrotic syndrome (SNSS) and 25% cases with steroid-resistant nephrotic syndrome (SRNS) and 11% congenital NS (CNS) that is known to be steroid-resistant. NPHS2 R229Q polymorphisms were determined by the polymerase chain reaction and RFLP technique utilizing specific primers.

Results: Mutation of NPHS2 R229Q gene was found in 6% of patients with NS, and in 17.64% of those with SRNS and 14.28% in CNS patients respectively. No mutation was found in control group.

Conclusion: The current research confirmed the association between NPHS2 R229Q gene polymorphism and lack of steroid responsiveness. NPHS2 R229Q gene mutation was found just in patient with steroid-resistance (CNS and SRNS). Further studies with a larger number of patients are needed.

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E-P03.30**Heterozygous versus homozygous phenotype in a novel MC4R mutation affecting a large consanguineous kindred**

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Heterozygous MC4R mutations are the most common known genetic cause of obesity, with more than 160 mutations described to date affecting 2–3% of the population in various cohorts tested. Homozygous or compound heterozygous MC4R mutations are much less frequent, and only few families have been described to date in which heterozygotes and homozygotes of the same mutation are found. We now describe large consanguineous inbred kindred with multiple individuals that are either homozygous or heterozygous carriers of the same novel MC4R mutation. We demonstrate that while all individuals with a homozygous MC4R mutation have morbid obesity, heterozygotes of the same mutation(s) have either a milder phenotype or no discernable phenotype, although on average they present with higher BMI.

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E-P03.31

High throughput sequencing identifies p.Y1435X as a novel truncating PKD1 mutation

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD), the commonest heritable renal disorder, is caused by mutations in *PKD1* or *PKD2*. *PKD1* mutations account for 85% of cases and cause more severe disease with earlier onset of end-stage renal disease (ESRD). Maltese ADPKD patients reach ESRD at an early age, generally between 40 and 50 years. **Materials and Methods:** Blood and saliva samples were obtained from a family with ADPKD. High throughput sequencing (HTS) was used to sequence the entire *PKD1* and *PKD2* coding regions extending up to 50 bp into the introns in each direction. SureSelect^{XT} Target Enrichment capture of a 2.6 Mb region, including *PKD1* and *PKD2*, was followed by sequencing on Illumina HiSeq4000. HTS data was mapped to GRCh37 as paired-end libraries using NextGENe software. A BED file was used to ensure mapping to *PKD1* and *PKD2* and excluding pseudogenes. To remove potential pseudogene contamination of data, the mutation list was filtered against an in-house database of 90 control HTS datasets and by pairwise blast of *PKD1* and relevant pseudogenes. **Results:** A heterozygous novel mutation in exon 15 of *PKD1*, c. 4305 C>G, p.Y1435X, was identified in the proband and his affected child but in none of the control datasets. The novel stop codon is in the 6th consecutive extracellular PKD

domain resulting in a truncated protein lacking a number of domains, including all the transmembrane domains. **Conclusion:** We report a novel, pathogenic, nonsense mutation in *PKD1* in a Maltese family. **Funding:** The NGS project: National Research and Innovation Programme 2014.

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E-P03.32

Hyperphagia questionnaire to evaluate the Prader Willi patients behavior related to food

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Background. Hyperphagia, one of the main clinical features of Prader Willi Syndrom (PWS), is a life-threatening symptom because it leads to morbid obesity and associated complications. **Aim.** To evaluate the eating behavior in PWS Romanian patients and to identify possible predictors for hyperphagia. **Material and methods.** The study included 11 PWS patients, 4 girls and 7 boys, mean age 25.37 ± 6.36 years for girls and 10.37 ± 6.77 years for boys, body mass index (BMI) 49.88 ± 4.95 kg/m² and 29.18 ± 16.53 kg/m² respectively. We applied a hyperphagia questionnaire with 11 items divided in 3 categories: Hyperphagic Behaviour (HB), Severity (HS) and Drive (HD). **Results.** HB represented 85.83% of quiz variance with a Cronbach’s Alpha Coefficient 0.88. We did not identify statistical significant differences between males and females for each separate question. The mean of cumulated answers for females was significantly larger than males. We identify a linear correlation between HB and BMI ($BMI = 9.51 + 2.25 * HB$) and also a linear correlation between BMI and age ($BMI = 14.40 + 1.40 * age$). We could predict the BMI depending on age and HB ($BMI = 0.9717 * age + 1.4713 * HB$). **Discussions.** The questionnaire evaluated important PWS eating behavior features like food stealing, eating from trash and waking up at night to eat. In our study, each additional unit of HB or age will increase the BMI with 2.25 units and 1.40 respectively. **Conclusions.** The weight gain could be predicted using age and some important eating behavior features. It is an important finding given the importance weight management for the syndrome. Results need to be verified on a larger cohort.

A. Dobrescu: None. **A. Chirita-Emandi:** None. **N. Andreescu:** None. **S. Farcas:** None. **M. Puiu:** None.

E-P03.33

Homozygosity for a double mutated AGXT allele in an Indian child with primary hyperoxaluria type 1

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Primary Hyperoxaluria (PH) is a rare autosomal recessive disorder commonly arising in childhood with nephrolithiasis, nephrocalcinosis, chronic renal failure. PH1 is the most common and severe form and it is caused by mutations in the AGXT gene. We report on a 2-year-old South Indian male referred to our centre for a clinical suspicion of PH, based on nephrocalcinosis, poor weight gain, high 24-hour urinary oxalate level (75.7 mg/24 h) and normal renal function. A third-degree consanguinity of the parents was reported. One elder sister died at 9 years for end stage renal disease due to nephrocalcinosis. Molecular analysis of the AGXT gene revealed two mutations both in homozygosity (c.32 C>G, p.Pro11Arg; c.167 T>A, p.Ile56Asn). An incomplete major haplotype was identified, due to mutation in Proline 11. In literature the p.Pro11Arg variant was reported in vitro to reduce the normal AGT activity to <2%. The p.Ile56Asn mutation produces an unstable enzyme with a reduced activity mainly on minor haplotype. This is the first case in which these two mutations are found on the same allele, which might be leading to a severe deficiency of enzyme activity and a more severe phenotype. In vitro studies of this double mutated allele could be useful to estimate the enzymatic activity of AGT and to understand how these two mutations could interact in a non-minor/non-major allele. Moreover, a double mutated AGXT allele advice a careful evaluation in carriers, since the identification of heterozygosity for two mutations could also lead to a wrong diagnosis of PH in them.

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E-P03.34

SLC22A12 and SLC2A9 mutations in Spanish patients with renal hypouricemia

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Introduction: Renal hypouricemia (RHUC) is a rare disorder characterized by impaired urate reabsorption in the proximal tubule. Patients present low serum levels of urate associated with excessive urate urinary wasting, and some have severe complications like exercise-induced acute renal failure or nephrolitiasis. Two types of RHUC have been reported; RHUC1, comprising most cases, is caused by mutations in the *SLC22A12* gene that encodes for the urate-anion exchanger URAT1, and RHUC2 is caused by mutations in *SLC2A9* encoding urate-hexose exchanger GLUT9. Only a few mutations have been detected in Europeans. Our goal was to identify mutations associated with RHUC in Spanish subjects. **Materials and Methods:** Fifteen patients presenting the clinical features of RHUC were included. Genomic DNA was isolated from blood. Coding exons of the two genes were amplified and analysed by PCR and automated sequencing. **Results:** Sequence analysis revealed mutations in each patient. Three *SLC22A12* mutations, c.1245_1253delGGCAGGGCT (p.L415_G417del), c.1400 C>T (p.A467 M) and c.1427 C>A (p.A476D), and one *SLC2A9* mutation, c.374 C>T (p.T125M) were identified. Only two patients presented the *SLC2A9* mutation. Missense mutation p.A476D, affecting transmembrane domain 11 of URAT1, is a novel mutation associated with RHUC1. **Conclusions:** Our study describes the clinical and molecular characteristics of the first Spanish RHUC patients. *SLC22A12* and *SLC2A9* mutation screening is an important evaluation for the diagnosis, and should be carried out in European patients suspected to have the disease. This work was supported by grant PI14/00760, co-financed by Instituto de Salud Carlos III (Spain) and the European Regional Development Fund “A way to build Europe”.

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E-P03.35**Dystonia, hypermanganesemia, polycythemia and chronic liver disease, caused by SLC30A10 mutation**

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Introduction: Mutation SLC30A10 causes abnormal manganese transport and accumulation of the manganese in the blood, liver and basal ganglia. It has been detected in patients with a complex clinical picture comprising irregular body movements, polycythemia, and hepatic fibrosis. About 20 patients have been documented so far. Here we present a child with early onset of the disease diagnosed after two decades of persistent symptoms.

Materials and Methods: Patient was followed up by pediatricians, gastroenterologists, hematologists and neurologists in the country and abroad without the precise diagnosis. Regular analyses of the blood counts, iron in the blood and liver enzymes, as well as MRI were performed.

Results: The onset of the disease was at the age of 2 years with progressive dystonia binding him to a wheelchair at the age of 10. At the age of 8 polycythemia was detected and treated by phlebotomies. Hepatic enzymes were increased, and cirrhosis was diagnosed during the adolescence. Final diagnosis was reached at the age of 30 years when the analysis of manganese in the whole blood showed elevation(499nmol/L). MRI of the brain showed hypersignal of the basal ganglia explaining neurological symptoms. Molecular analysis confirmed homozygous SLC30A10 mutation. Oesophageal varices were treated during three hospitalizations thereafter. Manganese chelation with disodium calcum edetate was planned accompanying oral iron supplementation which is expected to cause some regression of the liver disease, and improvement of dystonia.

Conclusions: We report a very rare clinical syndrome due to the SLC30A10 mutation. Specific therapy is available and will be implemented.

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E-P03.37**Mutations spectrum of ATP7B gene and two possible modifier genes in a Russian patients with Wilson Disease**

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Introduction: Wilson's disease (WD) is an inherited disorder of copper homeostasis characterized by abnormal copper accumulation. Clinical polymorphism may be associated with combination of several factors: the variety of gene ATP7B mutations, the influence of the external environment and the modifier genes. Materials and methods: In a pilot study we examined 11 WD families. We designed targeted panel NimbleGen SeqCap EZ Choice: 151012_HG38_CysFib_EZ_HX3 (ROCHE) for analysis full genes CFTR, ATP7B, HFE and APOE. SIFT, Polyphen-2, LTR, Mutation Taster algorithms were used to predict the effects of the mutations. All patients underwent full clinical examination according to EASL Clinical Practice Guidelines. Results: 14 distinct mutations in ATP7B were detected, 5 of which were novel. The most frequent mutation c. C3207A (p.H1069Q) in ATP7B was detected in 14 cases (6 homozygous and 8 heterozygous). Two patients also had homozygous and compound-heterozygous mutations in the HFE. Summary information is presented in the Table 1:

Conclusions: The pilot study confirms genetic heterogeneity of WD and the importance of studying the influence of modifier genes. The research will be continued in a larger group of WD patients. The study was implemented under the Russian Science Foundation grant №14-50-00069.

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Gene	Mutation (*-novel)	Number of identified alleles
<i>ATP7B</i>	c.C3207A(p.H1069Q)	20
	c.T205G(p.C69G)*	5
	c.C208A(p.Q70K)*	4
	c.C2078G(p.S693C)	4
	c.T2077G(p.S693A)	3
	c.C2332G(p.R778G)	2
	c.-128_-127insACCTC*	2
	c.G3190A(p.E1064K)	1
	c.C3955T(p.R1319X)	1
	c.G1603T(p.E535X)*	1
	c.197_198insCTTCACA:p.G66fs*	1
	c.G2605T(p.G869X)	1
	c.A3034T(p.I1012F)	1
	c.C4301T(p.T1434M)	1
<i>APOE</i>	c.T388C(p.C130R)	5
	c.C526T(p.R176C)	2
	c.A437C(p.Q146P)	2
<i>HFE</i>	c.C187G(p.H63D)	4
	c.G322C (p.E108Q)*	2
	c.A193T(p.S65C)	1
	c.G845A(p.C282Y)	1

E-P03.38

Clinical picture of homozygous c.1943G > A mutation in the *WFS1* gene in a girl with Wolfram syndrome

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Introduction: Wolfram syndrome is a rare progressive neurodegenerative disorder characterized by *diabetes mellitus* type I, diabetes insipidus, sensorineural deafness, bilateral optical atrophy and neurological signs (DIDMOAD). Its prevalence is reported 1:100 000 - 1:800 000. It is an autosomal recessive disease, there are two types described - type I is caused by mutation in *WFS1* gene (4p16.1), type II - mutation in *CISD2* gene (4q24). Heterozygous mutation in *WFS1* gene is described as a cause of autosomal dominantly inherited non-syndromic low-frequency sensorineural hearing loss. Materials and Methods: We present a 12 year old girl, born to non-consanguineous parents, from twin pregnancy. Her twin-sister is healthy. She was referred to hospital at age of 3 y 7mo due to

complications after varicella infection (vulvitis). At hospital *diabetes mellitus* type I, hypothyreosis, hydronephrotic transformation was diagnosed. At age of 7 megacystis, bilateral obstructive megaurether, poor weight gain and short stature, optic nerve subatrophy was diagnosed. At age of 12 diabetes insipidus was suspected. Pedigree - the proband's father has had repeated bilateral otitis media and hearing problems, maternal father has unilateral hearing loss. A molecular testing of *WFS1* gene was performed. Results: A homozygous pathogenic (class 5) c.1943G > A, p.W648* mutation was detected that confirms Wolfram syndrome. LOVD and PubMed database reveals c.1943G > A mutation only in compoundheterozygous state. Conclusions: We report a family with a child with Wolfram syndrome due to a homozygous c.1943G > A mutation in *WFS1* gene, and some possible *WFS1* gene mutation carriers with hearing problems.

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E-P04

Skeletal, connective tissue, ectodermal and skin disorders

E-P04.02 Bruck Syndrome in a Mozambican patient with a homozygous mutation in *FKBP10* gene

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Introduction: Bruck Syndrome (BS) is a rare autosomal recessive disorder that is phenotypically related to Osteogenesis Imperfecta (OI). It is clinically characterized by congenital joint contractures with pterygia, bone fragility, postnatal short stature, limb deformities and progressive scoliosis. BS is caused by variants in two genes: *FKBP10* and *PLOD2*. It has been proposed that mutations in *FKBP10* gene may be associated with a moderately severe OI phenotype in patients with BS type 1. Materials and Methods: We report a Mozambican female patient with a clinical diagnosis of BS due to a homozygous deletion in *FKBP10* gene. Results: The patient has congenital left coxa vara, mild contracture of the left knee and congenital bilateral flexion contracture of the elbows. She has short stature with an accentuated lumbar hyperlordotic attitude, a triangular face and small joint laxity. Until now, she has had three fractures of long bones. She had a mild motor

delay and her cognitive development is normal. The presence of congenital joint contractures and bone fragility lead to the clinical diagnosis of Bruck Syndrome. The sequencing analysis of *FKBP10* gene revealed a homozygous 1-base-pair duplication (c.831dup), which confirms the clinical diagnosis. Conclusions: There are seven other patients with this mutation in *FKBP10* gene described in the literature, two of which are from the same geographic area our patient. The confirmation of the diagnosis is important for the patient, allowing a better management of the disease and also for her family members at risk, enabling Prenatal Diagnosis and Preimplantation Genetic Diagnosis.

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E-P04.06

A case of hypochondroplasia with medial temporal lobe dysgenesis and early-onset seizures

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Background: Hypochondroplasia (HCH) is an autosomal dominant condition, clinically characterized by short stature, with relatively long trunk and short limbs, increased lumbar lordosis, occasional macrocephaly and frontal bossing. HCH has not been commonly associated with neurological problems. Some authors have reported increased prevalence of mental deficiency (up to in 9% of the patients). Patient: Our boy was admitted to the hospital at the age of 3 months with seizures, which were well controlled by anti-epileptic drugs. Magnetic resonance imaging showed temporal lobe dysgenesis. A clinical diagnosis of hypochondroplasia was hypothesized because of a disproportionately short stature with relative macrocephaly. Molecular analysis of the FGFR3-gen, detected a c.1620C > G mutation, resulting in p.(Asn540Lys). Conclusions: Recently several more cases of HCH due to FGFR3 (N540K) mutations with temporal lobe dysgenesis, accompanied by epilepsy were reported. These findings might highlight the importance of neurodevelopmental follow-up of these children, with a low threshold for neuroimaging. Prospective study of these data should elucidate how common these characteristics are in this group of patients.

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E-P04.07

Incidence of mutations in the *TNSALP*, *GGPS1* and *CYP1A1* genes in patients with atypical femur fractures

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Atypical femoral fractures(AFF) are uncommon and often related to prolonged bisphosphonate(BP) treatment. Isolated AFF cases are linked to mutations of Tissue-Nonspecific Alkaline Phosphatase(*TNSALP*), as a presentation of hypophosphatasia in adults. Mutations in the geranylgeranyl pyrophosphate synthase(*GGPS*) enzyme which can be inhibited by BPs, and in the enzyme of the cytochrome P450 superfamily(*CYP1A1*), related to the metabolism of several drugs, have also been associated with some AFF cases. Our aim was to analyse the clinical characteristics and incidence of *TNSALP*, *GGPS1* and *CYP1A1* gene mutations in AFF.

Methods: 17 women with AFF(aged 68 ± 10 years) were included. Sanger sequencing for the *TNSALP*, *GGPS1* and *CYP1A1* genes was performed in all patients. We analysed the ALP substrates(vitamin B6 and PEA), bone turnover markers, bone mass, previous antiosteoporotic treatments and the clinical characteristics of the patients and their fractures.

Results: 2/17 patients(12%) presented heterozygote mutations in *TNSALP*(p.G288A) and *CYP1A1*(p.R136H), respectively. The patient with the *TNSALP* mutation presented increased vitamin B6 and reduced serum ALP levels. The patient with the *CYP1A1* mutation had glucocorticoid-induced osteoporosis treated with BPs during only 3 years. All patients received BPs(94% alendronate) for 74 ± 45 months, and nearly 50% also received glucocorticoids. AFF were bilateral in 35% of cases and 76% had previous fragility fractures.

Conclusions: Mutations in the *CYP1A1* or *TNSALP* gene may be related to the development of AFF in some patients receiving BPs. Evaluation of ALP substrates in patients with low ALP levels allows the identification of hypophosphatasia. The role of *CYP1A1* mutations in AFF needs further study.

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E-P04.08**Association between a common variant near *LBX1* and idiopathic scoliosis in Bulgarian population**

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Introduction: Idiopathic scoliosis (IS) patients exhibit occurrence rates of somatosensory disorders higher than the general population. The *ladybird homeobox 1 (LBX1)* gene is essential for the survival of somatosensory neurons in the dorsal spinal cord, thus alterations in *LBX1* expression levels might play role in IS etiopathogenesis via somatosensory dysfunction. The purpose of our case-control study was to investigate the association between a common variant, rs11190870 (T/C), downstream of *LBX1* and IS in Bulgarian population.

Materials and Methods: The association study was performed on 105 patients and 210 controls after obtaining written informed consent. The mean Cobb angle was $54.6^\circ \pm 22.7$ and the mean age of the patients was 11.2 ± 3.1 years. The genotyping was carried out by TaqMan Real-Time PCR method. The statistical analysis was performed by Pearson’s Chi-squared test and Fisher’s Exact Test with p-value less than 0.05 as statistically significant.

Results: The frequencies of the T allele and the TT genotype in the total group of patients and in the subgroup of patients with Cobb angle above 40° were significantly higher than that in the controls ($p < 0.05$). In addition, this case-control study revealed statistically significant association between rs11190870 (T/C) and IS in females, adolescent idiopathic scoliosis (AIS), familial and sporadic IS cases.

Conclusions: The results confirmed previously reported associations between the common variant near *LBX1* and IS in Caucasian and Asian population groups and suggested that the molecular marker rs11190870 (T/C) is an independent predisposing and modifying factor of IS in Bulgarian population.

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E-P04.09**IL-27 gene structural variations in Behcet’s disease**

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Introduction: Behcet’s Disease (BD) is a chronic systemic inflammatory disease of unknown etiology, principally characterized by relapsing periods of a broad range of clinical symptoms. Cytokines play fundamental roles in the pathogenesis of BD. Polymorphisms within cytokine genes have been found to play a pathogenic role in the development of autoimmune/inflammatory disorders. Interleukin 27 (IL-27), a new pro/anti-inflammatory cytokine, is a great candidate for chronic inflammatory disease studies. The purpose of this study was to investigate a possible association between polymorphisms in the IL-27 gene and susceptibility to BD. **Materials and Methods:** Fifty Iranian patients with BD and one hundred healthy individuals were examined for rs153109A/G and rs181206T/C IL-27 gene single nucleotide polymorphisms using RFLP-PCR and ARMS-PCR, respectively. Allele and genotype distributions were compared between groups using chi-square or Fisher’s exact test. **Results:** Frequencies of the rs153109AA genotype and rs153109A allele were statistically higher in BD patients comparing with the control group ($p = 0.034$ and $p = 0.011$, respectively). The genotype and allele frequencies of rs181206 T/C polymorphism in BD patients were not significantly different from those of healthy controls. **Conclusions:** Present findings demonstrate for the first time that IL-27 gene rs153109 A/G SNP may be involved in susceptibility to BD in the Iranian population.

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E-P04.11**Two novel mutations of FBN1 in Jordanian patients with Marfan syndrome****Abujamous**

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Introduction: Marfan syndrome is an autosomal dominant inheritance disorder with a 1/5000-live-birth

prevalence. More than 3000 mutations have been characterized thus far in the *FBNI* gene. The goal of this study is to facilitate Marfan syndrome diagnosis in Jordanian patients using a molecular genetic testing. Material and Methods: All of the 65 coding exons and flanking intronic sequences of the *FBNI* gene were amplified using polymerase chain reaction and were subjected to sequencing in five unrelated Jordanian patients suspected of having Marfan syndrome. Results: Four different mutations were identified, including two novel mutations: the c.1553dupG frame-shift (p.Tyr519Ilefs*14) and the c.6650 G > A (p.Cys2217Tyr) missense mutations. Two other missense mutations, c.2243 G > A (p.Cys748Tyr) and c.2432 G > A (p.Cys811Tyr), have been previously detected. One patient was heterozygous for the synonymous substitution variant c.1875T > C (p.Asn625Asn; rs#25458). Additionally, eight variants in the intronic sequence of the *FBNI* gene were identified, of which the c.2168-46 A > G mutation was a new variant. The data provide molecular-based evidence linking Marfan syndrome to pathogenic mutations in the *FBNI* gene among Jordanians for the first time. Conclusion: we detected four *FBNI* mutations in four Jordanian patients. Two of these mutations were previously identified and two are reported for the first time, one is *de novo* and the other is familial. These results will facilitate the molecular genetic diagnosis of MFS in Jordan and potentially prevent disease complications on the basis of molecular diagnosis.

Abujamous: A. Employment (full or part-time); Modest; King Hussein Cancer Center.

E-P04.12

Metaphyseal Chondromatosis with D-2-Hydroxyglutaric Aciduria - A case report

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Normal 0 false false false EN-GB JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name: "Table Normal"; mso-style-rowband-size:0; mso-style-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0 cm 5.4 pt 0 cm 5.4 pt; mso-para-margin-top:0 cm; mso-para-margin-right:0 cm; mso-para-margin-bottom:10.0 pt; mso-para-margin-left:0 cm; line-height:115%; mso-pagination:widow-orphan; font-size:11.0 pt; font-family:Calibri; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin;

mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-ansi-language:EN-GB;} Metaphyseal chondromatosis with D-2-hydroxyglutaric aciduria (MC-HGA, OMIM 614875) is a rare entity comprising severe chondrodysplasia, organic aciduria, and variable cerebral involvement. Seven patients with MC-HGA have been described in the literature. In two patients mutations in IDH1 have been identified (p.R132H and p.R132S) as apparent somatic mosaicism. We describe the case of a 5-year-old boy who presents with MC-HGA. He was born term after an uncomplicated pregnancy with a BW of 2630 grams. He was subsequently found to have congenital heart defect. He required feeds by NGT. During the 1st year of life he developed epilepsy, disproportionate short stature, leg length discrepancy and scoliosis. He also presented with developmental delay. A skeletal survey identified significant metaphyseal dysplasia with irregularity in mineralisation of expanded, cup-shaped metaphyses with radiolucent non-ossified cartilage affecting knees, ankles, wrists, elbows, shoulders; the spine was normal. He suffers from pain and swelling in fingers. A marked isolated increase in 2-hydroxyglutarate has been identified. Genetic analysis identified a p.R132H mutation in IDH1. There was no evidence of mosaicism. Thus, this is the 2nd report of MC-HGA associated with the p.R132H mutation in *IDH1*. In view of the genetic analysis result and the clinical presentation, we believe that a high level mosaicism is possible, which we are planning on exploring further.

<!--EndFragment--> **A. Beleza-Meireles:** None. **M. Chung:** None. **M. Jansson:** None. **A. Calder:** None. **M. Irving:** None.

E-P04.14

Variable expressivity of the c.749 C > G mutation in the *FGFR3* gene in two unrelated families of Muenke syndrome

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Introduction: Muenke (OMIM#602849) syndrome is an autosomal dominant disorder characterized mainly by uni- or bicoronal synostosis, midfacial hypoplasia, hearing impairment, intellectual disability and the presence of the c.749 C > G (p.P250R) mutation of the *FGFR3* gene.

Reduced penetrance and variable expression contribute to wide spectrum of clinical findings of this syndrome.

Material and methods: 7 members of 2 unrelated families from East Aukštaitija region of Lithuania with the same mutation in the *FGFR3* gene display a wide spectrum of clinical features of Muenke syndrome. Two siblings from 1st family and proband from 2nd family have classical features (brachycephaly/plagiocephaly, frontal bossing, mid-facial hypoplasia, brachydactyly) of this syndrome. Other members of these two families show milder craniofacial phenotype compared to those mentioned above.

Results: Heterozygous mutation c.749 C > G, p.P250R (rs4647924, CM960655) in the exon 7 of the *FGFR3* gene was identified by Sanger sequencing and diagnosis of Muenke syndrome was confirmed in the three probands with classical features from both families. Later, the same mutation was also detected in all other members affected with Muenke syndrome of the two families.

Conclusion: These families illustrate variable expression of Muenke syndrome in association with the same mutation of the *FGFR3* gene.

A. Matulevičiene: None. **B. Burnytė:** None. **I. Kavaliauskienė:** None. **R. Meskiūnė:** None. **R. Matulevičiute:** None. **B. Aleksuniūnė:** None. **L. Ambrozaityte:** None. **A. Utkus:** None. **V. Kucinskas:** None.

E-P04.15

a novel missense mutation of the *tyr* gene in an Iranian patient with oculocutaneous albinism

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Introduction: Oculocutaneous albinism (OCA) is a heterogeneous group of autosomal recessive disorders caused by mutations of the tyrosinase (*tyr*) gene and defect in melanin synthesis which is characterized by reduced or completely absent pigmentation in both ocular and skin and hair. Case presentation: In the present study, we performed a targeted next-generation sequencing panel to screen for 16 genes in a patient with clinical symptoms of albinism from non-albinism parents. This identified one pathogenic mutation in the form of c.996 G > A (p.Met332Ile Het) and one novel missense mutation, c.902CC > G (p.Pro301Arg Het) on the *TYR* gene. The parents were tested for all of the

exon and intron flanking regions of the *tyr* gene by direct sequencing to validate the mutations. The father was a heterozygous carrier for c.996 G > A (p.Met332Ile), and the mother was a heterozygous carrier for c.902 C > G (p. Pro301Arg). Conclusion: The novel missense mutation c.902CC > G (p.Pro301Arg) on the *tyr* gene, was found on molecular genetic testing of this patient which in a compound-heterozygous with another mutation causes a lack of the tyrosinase enzyme and disturbs the melanin biosynthesis process.

F. Sadeghi: None. **H. Kahroba:** None. **M. Nemati:** None. **E. Sakhinia:** None.

E-P04.16

The search for genetic susceptibility markers of osteoarthritis in women with undifferentiated forms of connective tissue disease from Russia

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The study of polymorphisms rs1799750 (*MMP1*), rs35068180 (*MMP3*), rs2252070 (*MMP13*), rs63118460 and rs2276455 (*COL2A1*), rs143383 (*GDF5*), rs1544410, rs7975232, rs731236 and rs2228570 (*VDR*) was held in women with primary osteoarthritis (OA) and connective tissue dysplasia (CTD). We used DNA samples from 333 women aged from 18 to 61 years, mean age 48,4 ± 4,7 years. OA was diagnosed according to the criteria of the American Association of Rheumatology (1995). Availability of CTD was evaluated using phenotypic classifica-

Table 1

Sign	Locus, (gene)	Marker χ^2 , p, (OR (95% CI))
CTD in Tatar	rs35068180 (<i>MMP3</i>)	*5A*5 4,49; 0,03, (0,57;(0,33–A 0,98))
CTD in Russian	rs2252070 (<i>MMP13</i>)	*A*G 4,26; 0,03; (1,58; (1,01–2,47))
	rs2276455 (<i>COL2A1</i>)	*G*G 7,02; 0,008; (1,74; (1,18–2,58))
	rs1544410 (<i>VDR</i>)	*A*A 3,93; 0,04; (0,3; (0,13–0,98))
	rs7975232 (<i>VDR</i>)	*G*T 4,45; 0,03; (1,65; (1,0 –2,72))
OA in Tatar	rs143383(<i>GDF5</i>)	*C 4,24; 0,03; (1,36; (1,01–1,83))

tion by Kadurina TI (2008). The association of allele *rs1544410*G* of *VDR* gene with an increased risk of OA developing was found. Allele *rs63118460*T* of *COL2A1* gene was associated with increased risk of hip OA. The risk of generalized osteoarthritis developing was increased in carriers of genotype *rs143383*C*C* of gene *GDF5*, *rs1544410*G* allele and genotype *rs1544410*G*G* of *VDR* gene. The distribution of genotypes and alleles in women of different ethnicity is presented in Table 1.

Based on the data obtained by the method of multiple regression we developed a risk assessment model of OA development considering various localization. Evaluation was carried out using the ROC-analysis (Tab. 2).

A. Tyurin: None. **R. Khusainova:** None. **D. Shapo-**

Table 2

Model 1	Model 2	Model 3
Hip Osteoarthritis	Knee osteoarthritis	Generalized osteoarthritis
$\chi^2 = 16,93 P = 0,039$ AUC = 0,686	$\chi^2 = 23,43 P = 0,009$ AUC = 0,707	$\chi^2 = 41,89 P = 0,000079$ AUC = 0,842

valova: None. **L. Lukmanova:** None. **R. Davletshin:** None. **E. Khusnudinova:** None.

E-P04.18

Sweet's syndrome in a patient with compound heterozygous mutations in the Mediterranean fever gene (MEFV)

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Sweet's syndrome (SS) is a rare disorder that often occurs in association with other systemic diseases. The disorder is characterized by development of nonpruritic, painful erythematous plaques with pseudovesicles, pustules and rare bullae. SS consists of a triad of erythematous plaques infiltrated by neutrophils in association with fever and leukocytosis. The pathological features of SS involve the dermis. The treatment of choice are systemic corticosteroids. We present an unusual course of SS in a man who carries compound heterozygous mutations in the MEFV gene. A 38 year old man from Sephardic Jewish ancestry, had suddenly developed fever, malaise, artralgia and painful erythematous plaques with pustules and bullae on the upper extremities. Diagnostic evaluation included leucocytosis, elevated erythrocyte sedimentation and C-reactive

protein rate. The symptoms exacerbated despite treatment with systemic corticosteroids. Mutational analysis of the MEFV gene revealed compound heterozygous M694V and V726A mutations. Clinical improvement appeared after administration of colchicine. Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever with serosal inflammation. The FMF gene (MEFV) encodes the protein pyrin that plays an important role in modulating the innate immune response. MEFV mutations have been identified primarily in patients from Mediterranean populations. Sweet's syndrome has been described in a patient with classical FMF as a possible new cutaneous feature and has never been described as a presenting sign of FMF. We suggest that SS skin lesions might be an only cutaneous presentation of FMF.

M. Michelson Kerman: None. **C. Vinkler:** None. **D. Lev:** None.

E-P04.20

association of sepsis after burn injury with snp s at TLR4 in mexican population

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Although the understanding of postsurgical pathophysiology has grown in recent years, we still can't accurately identify patients with burns who have an increased risk of infectious complications and death. This inexplicable variation is probably influenced by heritable factors. Polymorphisms affecting Toll-like receptor (TLR) structure and function could be candidate variations associated with sepsis development, due to their essential coordinator role in innate immunity. The present study examined the correlation between 6 SNPs at the TLR4 locus and sepsis in 200 post-burn patients with burns of 15 percent of total body surface and 200 subjects from a Mexican healthy general population. This was genotyped using the 5' exonuclease assay. Then we classified in two groups: a) sepsis; and b) non-sepsis patients. Clinical data were prospectively collected and microbiological studies were performed for etiologic identification. We found that the rs2737190 polymorphism is associated with the presence of sepsis while the other 5 SNPs are not. We showed that the A allele frequency was 60% in sepsis group, with a value significantly higher than that observed in non-sepsis group ($p < 0.05$). The allele phenotype relationship was analyzed with the statistical method STRAT that considered population stratification and the results were adjusted with

potential confounders. Our results, implies that rs2737190 may be useful marker for the genetic study of sepsis.

C.A. colin: None. **R. franco cendejas:** None. **H. cortes:** None. **N. leyva:** None. **J.J. magaña aguirre:** None.

E-P05 Cardiovascular disorders

E-P05.02

Targeting sequencing in Russian families with arrhythmogenic right ventricular cardiomyopathy: first results

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Introduction: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a hereditary progressive cardiac muscle disease characterized by fibrofatty myocardial dysplasia, ventricular arrhythmia, and sudden cardiac death. In Russia, most studies are targeted at hypertrophic and restrictive cardiomyopathy, with insufficient data on ARVC. Materials and Methods: In this study we analyzed a cohort of 43 patients from 13 ARVC families. The diagnosis was established according to the Task Force Criteria of the European Society of Cardiology revised in 2010. The assay was performed on the MiSeq (Illumina, USA), panel TruSight Cardiomyopathy Target Genes. Coding regions of 46 genes associated with the development of inherited cardiomyopathies, including genes *PKP2*, *PLN*, *DSP*, *DSC2*, *DSG2*, *JUP*, *TMEM43*, *DES*, *TTN*, *LMNA* associated with the development of the hereditary form of ARVC were sequenced. Results: At present 35 patients from 10 families are tested. Pathogenic mutations were confirmed in 1 family. We have found new frameshift mutation c.355delT in the *PKP2* in the patient with a definite diagnosis of ARVC. Subsequent family assessment showed that all three of the proband's children also carried this mutation. Data of remaining 8 patients are processed. Current mutation detection rate was 1/10 (10%). Conclusions: The first results of our work suggest that Russian ARVC population is not

similar to same European populations. Mutation detection rate is significantly lower than expected. More comprehensive gene panel or exome sequencing is needed to explore new variants and genes potentially involved in ARVC pathogenesis. This study was supported by Russian Science Foundation grant №14-50-00069.

M.A. Fedyakov: None. **O.E. Veleslavova:** None. **S.V. Apalko:** None. **O.V. Romanova:** None. **N.Y. Shved:** None. **Y.V. Shubik:** None. **S.D. Rud:** None. **T.E. Ivaschenko:** None. **A.M. Sarana:** None. **S.G. Scherbak:** None. **O.S. Glotov:** None.

E-P05.03

The contribution of genetic variants to plasma lipid composition related to the atherosclerotic status

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Introduction. Lipid metabolism is determined by genes, which mutations can lead to modulation in enzyme activity with lipid profile change consequently. This study was carried out to estimate the contribution of lipid metabolism gene variants related to the atherosclerotic status and their association with lipid indicators in Russian population.

Materials and Methods. The study involved 124 people older than 55 years divided into 3 age- and sex-matched groups according to the values of intima - media thickness. Lipid profiling was measured by homogeneous enzymatic colorimetric test. SNPs in *ApoE* (rs769452), *APOC3* (rs5128), *LIPC* (rs2070895) and *LPL* (rs328) genes were detected by allele-specific real-time polymerase chain reaction method using commercial kits.

Results. The distributions of genotype and allele frequency between patients with atherosclerosis and the controls were equal for *ApoE*, *APOC3* and *LPL* genes polymorphism. Wildtype *LIPC* genotype was associated with increased risk of severe atherosclerosis development (OR = 2,88; 95%CI 1,02 - 8,13). *LIPC* –250A genotype homozygote carriers showed decreased total cholesterol and low density lipoprotein levels. Heterozygous carriers of *LPL* Ser447Ter polymorphism had lower atherogenic index than non-carriers. Heterozygous carriers of *ApoE* Leu28Pro polymorphism had lower triglycerides blood level than non-carriers. No associations between *APOC3* genotypes and blood lipid levels were observed.

Conclusion. Natural polymorphic genes variants with protective effect such as *LIPC* –250A could be a prototype

for the predictive gene therapy of multifactorial diseases including atherosclerosis. This research was supported by the Russian Science Foundation grant No: 15-15-10022

E.V. Butenko: None. **S. Timofeeva:** None. **E. Derevyanchuk:** None. **T. Shkurat:** None.

E-P05.05

Next generation sequencing revealed new mutations in Bulgarian patients with hyperthrophic cardiomyopathy

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Background: Hyperthrophic cardiomyopathy is the most common genetic heart disorder, affecting 1 in 500 people worldwide. It is characterized by inappropriate myocardial hypertrophy that occurs in the absence of an obvious inciting hypertrophy stimulus. The genetic basis of the disease include defects in several of the genes encoding for the sarcomeric proteins, such as myosin heavy chain, actin, tropomyosin, and titin. With the advances of genomic technologies multiple mutations have been identified, with genotype-specific risks for mortality and degree of hypertrophy. Materials and methods: We have analysed 5 patients meeting criteria for familial hyperthrophic cardiomyopathy by using next generation sequencing of panel of genes connected to cardiac function. First, we filtered the detected gene alleles based on their frequency according to the existing data bases - ExAC_all, ExAC_NFE, 1000g_all, 1000g_eur. Then six variant prediction programs were used for estimation of non-annotated genetic variants discovered

in the genes - ClinVar Effect, SIFT, Polyphen2 HDIV, Polyphen2 HVAR, MutationTaster, FATHMM. Results: In 4 of 5 analysed patients (80%) we found new genetic variants predicted as pathogenic from at least 3 prediction programs, they are presented in the following table:

Conclusion: Gene panel analysis by next generation sequencing becomes gold standard in evaluation of genetic basis for hyperthrophic cardiomyopathy. Data collection and clinical follow-up will allow us to improve prognostic risk stratification in our patients.

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E-P05.06

Study of polymorphic genes relevant to folic acid metabolism in pregnant women from West Ukraine with prenatally diagnosed fetal heart defects

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The purpose of study was to investigate genetic variations in MTHFR and MTHFD1 genes in pregnant women from West Ukraine in cases of prenatally diagnosed fetal heart defects. Genotyping of *MTHFR* 677 C>T, *MTHFR* 1298 A>C and *MTHFD1* 1958G>A polymorphisms was performed in 35 pregnant women with prenatally diagnosed congenital heart defects in fetus and in 30 healthy women. The molecular genetic analysis was performed by PCR and RFLP. The frequency of mutant *MTHFR* 677 T allele was significantly higher among the women with fetal heart defects comparable to controls (47% and 28% respectively, $p = 0.04$), while the frequency of mutant *MTHFR* 677 TT genotype was almost identical (14% and 10% respectively, $p = 0.27$). Probable cause of revealed difference appeared to be a significant increase in the frequency of heterozygous carriers of mutant *MTHFR* 677 T allele among the women with fetal heart defects (65,7% and 36,7%, $p = 0,04$; [OR = 3.31 (95% CI: 1.07 - 10.51)]. The frequency of *MTHFR* 677CC genotype was significantly more often in controls (53% and 20%; $p = 0.02$), that is associated with 4-fold decreased risk to having offspring with congenital heart defects [OR = 0.22 (95% CI: 0.06 - 0.74)]. In cases of *MTHFR* 1298 A>C and *MTHFD1* 1958G>A polymorphisms the frequencies of alleles and genotypes were not significantly different in women with fetal heart defects comparable to controls ($p > 0,05$). In conclusion, the carrying of *MTHFR* 677 T allele seems to be highly probable risk factor for Ukrainian women to have a child with

Gene	Location	Aminoacid and nucleotide change
PATIENT1		
MYH7	chr14:23893238exon23:c.G2800A:p.A934T	
PATIENT2		
MYBPC3	chr11:47364607exon14:c.G1316A:p.G439D	
MYBPC3	chr11:47369975exon6:c.G772A:p.E258K	
PATIENT3		
MYBPC3	chr11:47353740exon32:c.C3697T:p.Q1233X	
PATIENT5		
ACTN2	chr1:236906268exon11:c.C1180T:p.R394W	

congenital heart disease, whereas *MTHFR* 677CC genotype provides credible protective effect.

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E-P05.07

Application of comparative genomic hybridization in patients with syndromic congenital heart disease

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Introduction: Congenital heart disease (CHD) is the most common birth defect and is a major cause of infant morbidity and mortality. The genetic background of syndromic CHD is very heterogeneous, chromosomal aneuploidy, Mendelian disorder and DNA copy number variations (CNVs) are responsible for the phenotype. The objective of our study was to evaluate the usefulness of whole genome array comparative genomic hybridization (aCGH) for detection of submicroscopic chromosomal aberrations in syndromic CHD cases, and to explore the relationship between the CNVs and CHD. Materials and Methods: We used Cytoscan 750 K oligonucleotide array (Affymetrix) to study 7 patients with mild to severe CHD phenotype and multiple congenital anomalies. The identified CNVs were confirmed by fluorescence in situ hybridization and G-banding. Results: Clinically significant copy number variations were found in 4/7 cases with size ranging from 1.36 Mb to 34.58 Mb. Two patients had CNVs associated with known syndromes: 8p23.1 microduplication and Cri du chat syndrome. Two cases presented rare genomic aberrations: unbalanced subtelomeric translocation t(4;6) (q33;q25) causing partial deletion of 4q34.3 and duplication of 6q25.1; microdeletion of 12q24.1. These genomic imbalances contain genes that have been associated with human CHD before. Conclusions: Array CGH has led to an increased detection of causal chromosomal imbalances in individuals with syndromic CHD. The results emphasize the growing importance of using genome-wide studies in patients with CHD to increase the number of genomic data associated with CHD and improve the CNV-phenotype correlations. This study was supported by the Ministry of National Economy, Hungary (GINOP-2.3.2-15-2016-00039).

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E-P05.08

Adiponectin expression in the epicardial adipose tissue of coronary artery disease patients

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Current studies indicate that epicardial adipose tissue (EAT) can play an important role in the development and progression of coronary atherosclerosis. Anatomical and functional relations EAT, coronary artery and the myocardium allow interaction and metabolism between these structures. EAT produce a number of bioactive substances adipokines including Adiponectin (ADIPOQ) involved in inflammation, atherogenesis and endothelial dysfunction. The aim of this study was to investigate an association of ADIPOQ expression level in EAT with severity of coronary atherosclerosis and anthropometric characteristics (BMI and waist circumference) in patients with coronary artery disease (CAD). Epicardial adipose tissue samples were obtained from 39 patients with CAD who underwent elective coronary artery bypass graft surgery. The degree of stenosis was confirmed by coronary angiography. The levels of mRNA ADIPOQ were measured by qPCR, protein levels of adiponectin were defined by western-blot. Real-time PCR was performed in CFX96 detection system with two reference genes (ACTB and RPLPO). Correlations between mRNA and protein levels of ADIPOQ and severity of coronary atherosclerosis, BMI and waist circumference were not found. Our data indicate that adiponectin EAT expression level is not associated with severity of coronary atherosclerosis.

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E-P05.09**Functional evaluation of the interaction between cigarette smoking and the coronary artery disease risk gene ADAMTS-7 in *Drosophila melanogaster***

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Coronary artery disease (CAD) is a complex disease resulting from an interplay of lifestyle and genetic causes whereby cigarette smoking has been shown to be one of the strongest lifestyle risk factors. Previously, CAD genome-wide association studies (GWAS) have proven that gene-smoking interactions are partly mediated by **ADAMTS-7, a CAD-risk gene**. Particularly, the allelic variation at rs7178051 that **associates with reduced ADAMTS-7 expression conferred stronger CAD protection in “never-smokers” compared to “ever-smokers”**. The loss of CAD protection in smokers is likely due to the induction of ADAMTS-7 expression in the vasculature by cigarette smoking. **ADAMTS-7 is a member of the “A disintegrin and metalloproteinase with thrombospondin motifs” (ADAMTS) family**, which plays a crucial role in **neointima formation and vessel stenosis**. Thus, a detailed understanding of the ADAMTS-7 CAD risk gene is of significant importance. We studied previously **smoking exposure in *Drosophila***. Interestingly, our RNA-seq analysis results revealed high expression of the ADAMTS-7 homolog in the tracheas of smoke-exposed flies, indicating that it might play an important role in this gene-smoking interaction. **The fruit fly consists of a primitive vascular system in comparison to other invertebrate models and the molecular mechanisms that regulate the formation of the tracheal tube seem to be similar to those involved shaping the vascular tube in mammals**. Therefore our main objective is to unravel the **physiological role and function of ADAMTS-7 in *Drosophila melanogaster*** and specifically to understand the interconnection between cigarette-smoking and the CAD-risk variants at the ADAMTS-7 risk locus observed in humans.

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E-P05.10**Distribution of Common TP53 and MDM2 Variants Among Down Syndrome Patients with Cardiac Abnormalities**

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Introduction: Cardiac abnormalities are common in people with Down syndrome (DS), Trisomy 21, and are mostly responsible for DS morbidity and mortality. The genetic mechanism of cardiac involvement has not fully understood but genes located in both chromosome 21 and other chromosomal regions are continuing to be investigated. To understand whether TP53 gene, which is involved in cardiac development, has a role in clinical presentation of DS, nucleotide variants associated with TP53 functions were investigated in this study. TP53 gene codon 72 Arg > Pro variant is commonly associated with TP53 protein functions. The MDM2 protein, which involved in TP53 protein degradation, is also reported to be regulated by the nucleotide variant within its first intron. In this study, allele distributions of these variants were assessed in DS patients with cardiac abnormalities.

Materials and Methods: DS patients with or without cardiac involvement and control subjects without cardiac abnormalities were included in this study. TP53 gene p. Arg72Pro (c.C21G) variant and MDM2 gene c.14 + 309 T > G variant was determined with melting analysis of hybridization probes.

Results: The Pro allele frequency of TP53 gene was about 0.58 in controls and DS (-) cardiac abnormalities. However, DS patients with cardiac abnormalities were having high frequency (0.66) of Pro allele. For MDM2 gene, controls and DS (+) cardiac anomalies were displayed similar frequency, but G allele was high (0.60) among DS (-) cardiac abnormalities.

Conclusion: The results are implying that the nucleotide variants regulating TP53 protein activities may be involved in DS clinical presentation associated with cardiac abnormalities.

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E-P05.11**Genetic variation in *DSP* gene in patient with ARVC/D and prolongation of QT interval**

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Introduction: The *DSP* gene encodes a protein which is called desmoplakin. This protein is an important component of desmosome structure in cardiomyocytes. Mutations in *DSP* gene, as one of known genes are responsible to Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D) and pattern of inheritance is autosomal dominant. ARVC/D has a wide spectrum of clinical manifestations. Patients with ARVC/D often have arrhythmias which can increase the risk of sudden cardiac arrest. Materials, methods and results (Case report): 20-year-old women, hospitalized at our center with a history of recurrent fainting attacks. Result of blood test was normal. History of sudden death under 45 year-old was positive in her family. QT interval prolongation (>500 msec) was calculated in electrocardiogram. Echocardiography was done and Right ventricular outflow tract (RVOT) enlargement was detected. All responsible genes for ARVC/D and LQT syndrome were studied by Next Generation Sequencing (Hiseq - Illumina platform) to confirm dual diagnosis. Only a reported variation (p.R1775I) was found in *DSP* gene and it is located in Rod domain of protein (Bauce et al, 2005). Bioinformatics tools have predicted that mentioned variation was deleterious. This nucleotide change was not detected in 100 ethnically matched healthy control subjects. Evidence of pathogenicity of p.R1775I has not been known yet and it is reported as uncertain significance. Conclusions: Overlapping characteristics of prolongation QT interval (LQT syndrome) and ARVC/D are described in this study and are related to *DSP* gene. The relation between role of *DSP* gene and prolongation of QT interval needs more attentive follow-up and studies.

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E-P05.14**Mutations in genes encoding sarcomeric and non-sarcomeric proteins in hereditary cardiomyopathies**

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Hereditary cardiomyopathies are common genetic disorders of the myocardium associated with structural and/or functional abnormalities. There are four major forms of cardiomyopathies according to the phenotype: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC). We developed a new next generation sequencing (NGS) panel as a diagnosis tool for genetic cardiomyopathies, using the TruSeq Custom Amplicon Low Input technology (Illumina). This panel includes the most common gene coding for cardiac sarcomeric proteins (*MYBPC3*, *MYH7*, *TNNI3*, *TNNT2*, *TPM1*, *MYL2*, *MYL3*, *ACTC1* and *CSRP3*). We also included other genes known to cause cardiac phenotype: *GLA*, *TTR* and *LMNA*. After preparation of the library, the target regions were amplified and sequenced on a Miseq^{MD} sequencer (Illumina). Bioinformatics analyses were performed using Miseq Reporter, SeqNext (JSI Medical Systems) and Smaug (home-made pipeline). All mutations and variants of unknown significance (VUS) were confirmed by Sanger sequencing. A total of 91 DNA from patients with different forms of cardiomyopathies were analyzed. The coverage of all target regions was satisfactory for all patients. A total of 45 probably pathogenic mutations were identified including 2 novel mutations. This method allowed molecular diagnosis in 40% of patients. 72.5% of causative mutations were located in genes encoding sarcomeric proteins vs 27.5% in non-sarcomeric. Although sarcomeric genes are frequently involved in hereditary cardiomyopathies, the clinical and paraclinical context should encourage in some cases to explore non-sarcomeric genes. This emphasizes the need of sustained clinico-biological discussions to orientate the genetic investigations in these patients.

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E-P05.15**Digenic heterozygous occurrence of two novel variants detected by next generation sequencing in patient with hypertrophic obstructive cardiomyopathy**

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Introduction: Cardiomyopathies are diseases of myocardium associated with cardiac dysfunction (WHO, 1995). Hypertrophic cardiomyopathy (HCM) is a primary, genetic cardiomyopathy resulting in enlargement of part of the myocardium.

Materials and methods: We report on 4 years old girl with sporadic form of asymmetric obstructive HCM without heart failure. Diagnosis was confirmed by catheterization when hemodynamically significant gradient from 25 to 80 mm was measured in the left ventricular outflow tract (LVOT) during sinus rhythm with change in pressure after extrasystole-drop in aortic pressure and rise in gradient to 100 mm (positive for Brockenbrought-Braunwald sign). Targeted NGS of 176 genes using TruSight Cardio gene panel (Illumina) was performed on DNA from the index patient. Results were validated by Sanger sequencing and segregation analysis in the affected family was performed.

Results: Two novel variants were detected in genes *CACNA1C* (p.Arg514Gly) and *SCN5A* (p.Arg800His), both heterozygous, connecting the clinical symptoms to digenic inheritance. Calcium channels that contain the alpha1C subunit play role in coupling the excitation and contraction in the heart. *SCN5A* codes the alpha subunit that mediates the permeability of the cell membranes for sodium ions regulated by levels of calcium. Each parent is found to be heterozygous carrier of only one of the variants. No other known pathological variants associated to HCM were found.

Conclusions: Although most familial cardiomyopathies are monogenic disorders, this study underlined digenic inheritance of HMC associated with novel variants, clearly indicating that due to diversity of the phenotype many genes remain to be identified.

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E-P05.16**Hypoplastic right ventricle, dysmorphic features and brain structural anomalies in a patient with a de novo 1p36.33p36.32 deletion**

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Monosomy 1p36 is the most common terminal deletion syndrome in humans. The phenotypic expression of patients with monosomy 1p36 varies according to the size of the deleted chromosomal fragment and is characterized by developmental delay, neuropsychiatric abnormalities, dysmorphic features, and congenital anomalies. Congenital heart defects are common features in patients with 1p36 deletions. A newborn is presented with chromosomal abnormality, severe dysmorphic features, and congenital heart defects. The proband is the first-born female child of non-consanguineous parents, from complicated pregnancy with polyhydramnion. The patient was consulted by clinical geneticist due to multiple congenital anomalies, and distinct dysmorphic features, including microbrachycephaly, straight eyebrows, deeply set eyes, wide and prominent nasal bridge, microstomia, large anterior fontanel, small posteriorly rotated, low set abnormal ears, pointed chin, expressed hirsutism was determined. CT scan revealed corpus callosum dysplasia, hydrocephaly and aqueductus stenosis. Hypoplastic right ventricle with atrial and ventricular septal defects and patent ductus arteriosus was detected by echocardiography. SNP array analysis identified a *de novo* 1p36.33p36.32 deletion, 12,6 Mb in size. Parents' conventional karyotype analysis confirmed *de novo* chromosomal aberration in the patient. The deletion region encompasses 128 OMIM genes, including *DVL1*, *GABRD*, *MMP23B*, *SKI*, *PRDM16*, *KCNAB2*, *RERE*, *UBE4B*, *CASZ1*. Haploinsufficiency of *DVL1*, *SKI*, *PRDM16*, *RERE*, *CASZ1* genes is likely to contribute to cardiovascular phenotype of the presented patient, whereas haploinsufficiency of *MMP23B* could be responsible for late-closing

anterior fontanelles. These results findings are consistent with previously reported studies, though hypoplastic right ventricle is a rare cardiovascular phenotype of 1p36 deletion syndrome.

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E-P05.17

Moderating effect of ppar -gamma on the association of C-reactive protein and ischemic stroke in patients younger than 60

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AIM: Previous studies has shown that inflammatory processes and modifiable risk factors, including hypertension, smoking, physical activity, diabetes, hyperlipidaemia, interact with genetic factors and contribute to stroke development. Peroxisome proliferator activated receptor- γ (PPAR γ) is important protein that plays a significant role in different processes and its gene variability could be considered a predictive marker for ischemic stroke. The aim of this study was to evaluate the moderating effect of PPAR- γ gene Pro12Ala variants on the association of C-reactive protein (CRP) and ischemic stroke (IS). **MATERIAL AND METHODS:** A total of 114 patients with IS and 135 healthy controls were included. The study group was selected among patients admitted to our hospital under clinical presentation of ischemic stroke, fulfilling the following enrolment criteria: 1) age 18–59 years, 2) Computed Tomography (CT) proven ischemic cerebral infarction. Exclusion criteria were: 1) diabetes mellitus in earlier medical history, 2) haemorrhagic cerebral infarction on CT scan, 3) age > 59. Blood samples for biochemical analyses (total cholesterol, triglycerides-TG, low-density lipoprotein - LDL, high-density lipoprotein-HDL, CRP) were collected after overnight fasting and analysed by using routine laboratory methods. **RESULTS:** In participants with PPAR CG or GG genotype CRP and IS were not statistically significantly associated (adjusted OR = 1.00; 95% CI 0.90–1.10; P = 0.933), but in participants with PPAR CC genotype, the association of CRP and IS was clear (adjusted OR = 1.67; 95% CI 1.21–2.31; P = 0.002). **CONCLUSION:**

PPAR γ had statistically significant moderating effect on association of CRP and IS in patients younger than 60. **A. Bazina:** None. **K. Starčević:** None. **J. Sertic:** None.

E-P05.18

Prevalence of dilated cardiomyopathy in the Russian patients with Marfan Syndrome

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Introduction. Usually dilated cardiomyopathy (DCM) in patients with Marfan syndrome (MFS) is explained by volume overloading due to aortic or mitral valve regurgitation, and progressive aortic root dilatation. Recent studies of J.R.Cook et al. “Abnormal muscle mechanosignaling triggers cardiomyopathy in mice with Marfan syndrome” have shown that fibrillin-1, a structural component of the architectural ECM, is a force-transmitting molecule protecting the mammalian heart against cardiomyopathy.

Materials and methods. We have performed clinical (Standard and 24-hours Holter ECG, EchoCG, Dopplerography, chest radiography) and genealogical examination of the 40 probands with MFS, 6 of them were children. All probands had met the Ghent’s criteria for MFS. Affected relatives were found in 13 families. Mutational screening in the *FBNI* gene was performed by PGM Ion Torrent followed capillary Sanger sequencing.

Results. We had detected 30 mutations in the *FBNI* gene in 31 (77,5%) index cases. Cardiomegaly was found in 30 (88%) adult probands and in 2 out of 6 children. All heart chambers enlargement, increased end-diastolic volume of the LV and reduced ejection fraction were found in 15 (37,5%) index cases.

Conclusion. The prevalence of DCM is very high in probands with MFS. The influence of this complication for the long-term prognosis in this clinical group might be underestimated. We suggest that mutations in the *FBNI* gene may play a direct causative role in cardiac remodeling in MFS patients. This work was supported by grant RNF № 16-15-10421. **V. Rumyantseva:** None. **U. Rogozhina:** None. **A. Bukaeva:** None. **E. Charchyan:** None. **E. Zaklyazminskaya:** None.

E-P05.19**Circulating miR-208b and miR-499 as potential and emerging biomarkers for diagnosis of Acute Myocardial Infarction**

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Background: Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality worldwide. Last years, several studies indicated that microRNAs (miRNAs) circulating in serum or in plasma are attractive biomarkers for several human diseases and altered circulating miRNAs expressions have been reported in AMI. The objective of the present study was to examine the expression of the miRNAs, miR-208b and miR-499, in AMI patients and to investigate whether these miRNAs could be useful biomarkers for AMI. Methods: AMI patients ($n = 50$) and healthy controls ($n = 50$) were retrospectively recruited for a comparison of their plasma miR-208b and miR-499 expression levels using TaqMan® MicroRNA assays. Results: Our results indicate that both miR-208b and miR-499 levels were significantly overexpressed in AMI patients compared to controls. The miR-208b and miR-499 levels in plasma were increased by 256-fold and 675-fold, respectively. Furthermore, ROC curves analysis demonstrated that circulating miR-208b (AUC: 0.9996) and miR-499 (AUC: 0.9992) levels are more informative for AMI diagnosis than cTnT (AUC: 0.9400) in AMI patients. Conclusions: Both these two studied microRNAs, miR-208b and miR-499, could be used as potential diagnostic biomarkers for AMI.

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E-P05.20**Mitochondrial DNA polymorphism can modify risk of atherosclerosis and its complications**

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Mitochondrial DNA is highly polymorphic in human populations, and there is some evidence that its polymorphism contributes to susceptibility to common diseases. We have genotyped mtDNA in several groups of patients with coronary and carotid atherosclerosis, including group of patients with early cardiovascular death (age below 55 years), as well as common population sample, and healthy people older than 68 years with no signs of atherosclerosis. Chi-square statistics was used to compare mtDNA haplogroups frequencies between the groups, and the differences with $p < 0.05$ were taken as significant. We found that mitochondrial haplogroup H1 (with population frequency 11.5%) had been more abundant in the patients with early cardiovascular death (21.3%), and with recurrent cardiovascular events during one year follow-up after infarction (20%). On the contrary, haplogroup J had higher frequency among healthy elder people (17.7%) comparative to the patients with atherosclerosis (4.6%) and common population (6%). In addition, T16189C polymorphism was more frequent in the patients with the first infarction before age of 55 (24%) comparatively to patients with later first infarction age (12.5%). Haplogroups H and J are known as having high (H) and low (J) OXPHOS efficiency and reactive oxygen species production. T16189C was associated with diabetes in some previous studies, and presumably can influence mtDNA copy number through altering mtSSB binding. Since oxidative stress is one of important factors for atherosclerosis development, the haplogroup differences in ROS production may contribute to development of atherosclerosis and its complications. The study was supported by RFBR grant no. 16-04-01481-A.

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E-P05.21**Utility of targeted next generation sequencing, comprising exonic regions with disease-causing variation, in a representative Czech cohort of paediatric and adult patients with hereditary arrhythmic syndromes**

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Introduction: Arrhythmic syndromes are rare, mostly autosomal dominant disorders with a high risk of severe ventricular arrhythmias and sudden cardiac death. We aimed to assess the utility of clinical exome(CE)-NGS (TruSight ONE; Illumina on HisSeq2500) in a representative cohort of Czech patients with clinical diagnosis of long QT-, catecholaminergic polymorphic ventricular tachycardia-(CPVT) and Brugada (BS) syndromes.

Material and Methods: A total of 28 cases (22/28 LQT, 3/28 CPVT and 3/28 BS) were studied. All patients and their families had undergone clinical genetic counselling, and 1st degree relatives were referred to cardiologic examination. ACMG.net variant pathogenicity classification was used.

Results: In the LQT cohort the detection rate was 73% (16/22). Highly likely pathogenic variation (PV) was found in *KCNH2* and *KCNQ1*, in 5/22 cases, while 2/22 patients had PV in *SCNA5A* and *RYR2* each. Most of the PV was of missense type (Classes 4–5). One case had PV in *KCNJ2* and 2 patients in *RYR2*. Interestingly, no PV was found in Brugada syndrome. All CPVT patients carried missense *RYR2* PV, classes 4–5. Class III cases are still being clinically examined to verify their clinical phenotype.

Conclusions: We report a high detection rate of PV (classes IV-V) in patients suffering from LQT- (73%) and CPVT- (100%) syndromes. Although the scope of PV in CE-NGS is adequate for the detection of PV in “channel disorders”, it fails to detect PV in other genes related to cardiological disorders. Supported by: 00064203, CZ.2.16/3.1.00/24022, 15-27682 A and NF-CZ11-PDP-3-003-2014.

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E-P05.22

PHACTR1 gene is associated with susceptibility to coronary artery disease and myocardial infarction in Bulgarian population

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A meta-analysis of replication case-control studies has shown significant evidence for association of the polymorphic variant rs9349379 in PHACTR1 gene with arterial stenosis. The aim of this study was to investigate the possible association between the polymorphism rs9349379 (PHACTR1) and the risk of coronary artery disease (CAD) and myocardial infarction (MI) in Bulgarians. A total of 820 subjects were included in the analysis. Of them, 324 were patients with angiographically documented CAD (199 with MI and 125 without MI) and 496 were population-based controls. The rs9349379 in PHACTR1 was genotyped by TaqMan SNP Genotyping Assay. Non-adjusted χ^2 -based analysis was applied for evaluation of PHACTR1 genotype and allele association with CAD and MI by PLINK 1.07. The rs9349379 allele C showed association with an increased risk of both CAD (OR1.33, CI95:1.01–1.74, $p = 0.046$) and MI (OR1.28, CI95:1.01–1.63, $p = 0.037$) and this trend was higher in the men's group (OR1.45, CI95:1.07–1.97, $p = 0.017$). As regards the risk of MI in the background of cardiac ischemia, the incidence of rs9349379 allele G was higher among patients with CAD without MI compared to patients with CAD who have experienced MI (44.59% vs 36.58%). These findings determine the protective role of allele G in stressful complications of already established CAD (OR0.71, CI95:0.52–0.99, $p = 0.046$). The contribution of PHACTR1 to the risk of developing cardiovascular complications still remains unclear and it has yet to be clarified by further genetic and functional analyses. Acknowledgements: This work was supported by Grant №26/27.05.2016 and DUNK01/2/28.12.2009 “National University Complex in Biomedical and Translational Research”, funded by National Science Fund, Ministry of Education, Youth and Science, Bulgaria.

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E-P05.24

Genetic effects of *PPARG1C* and *TNF1* gene variants related to cardiovascular disorders

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Introduction: Study was aimed to investigate the associations between the SNPs in *PPARGC1A* (Gly482Ser), *TNF* (-308G-A) genes and coronary heart disease (CHD) and hypertension and their relations to leukocyte profile and plasma lipid composition in Russian population. Materials and Methods: 124 participants older than 55 years were divided into groups according to the anamnesis data. All participants were genotyped for SNPs *PPARGC1A* (Gly482Ser) and *TNF* (-308G-A) by allele-specific real-time polymerase chain reaction method using commercial kits. Lipid profiling was measured by homogeneous enzymatic colorimetric test. Results: The results of this study suggested the presence of the mutation *PPARGC1A* (Gly482-Ser) increases the risk of coronary heart disease by 2.4 times (OR 1.17–4.97), but is not associated with arterial hypertension. *TNF* -308A t allele has demonstrated a protective effect, reducing the risk of developing hypertension (OR 0.01–0.84), but is not associated with CHD. The relationship between genotypes and the average values of leukocyte and lipid profiles remained non-significant in our population. However the heterozygous genotype of *TNF* was related to an increased level of drumstick neutrophil, compared to the control group. Conclusion: Thus, the investigated polymorphic variant (Gly482Ser) of *PPARGC1A* gene is a reliable marker of CHD, whereas in the case of hypertension the association with (-308G-A) of *TNF* gene was identified. This research supported by the grant of the Russian Science Foundation №. 15-15-10022.

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E-P05.25

Polymorphisms of TNFA in hypertensive patients

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Introduction: Hypertension is a complex, multifactorial and polygenic disease. This descriptive study analyzed whether there are differences in TNFA -238 G>A and TNFA -308 G>A polymorphisms that encode molecules involved in inflammation among hypertensive patients, refractory hypertensive patients and controls. **Materials and methods:** We performed a case-control study with 444 subjects: 234 hypertensive patients (HTA), 50 refractory hypertensive patients (HTA-R) and 160 controls. The DNA was amplified by PCR and TaqMan trials allowed allelic discrimination. There were no statistically significant differences in either age or sex between all 3 study groups. **Results:** In the analysis of the polymorphisms of TNFA -308 G>A, the distribution of the allele AA for HTA and HTA-R groups was 0% and 2.4%, respectively ($p = 0.02$). No other significant differences were found in the study groups or in the analysis of TNFA -238 G>A genotypes. **Conclusion:** Variations in the polymorphism TNFA -238 G>A do not affect the chances of having refractory hypertension. For the first time, we describe the association between the AA genotype of TNFA -308 G>A and refractory HTA. Individuals carrying the allele A will have an increase in the levels of TNFα which causes endothelial damage. This endothelial alteration could justify the severity of hypertension and the poor response to drugs. This could lead to future therapeutic opportunities in these patients.

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E-P05.26

SERPINE1 gene polymorphic variant in the predisposition to cardiovascular diseases

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Genes of predisposition to cardiovascular diseases are actively studied worldwide, including the plasminogen activator gene (*SERPINE1*). However, its role in the development of hypertension, atherosclerosis, coronary artery disease, myocardial infarction is poorly understood, and the results of the studies tend to be contradictory. In this regard, the aim of this work was to analyze the association of *SERPINE1* gene polymorphism (-675 5 G/4 G, rs587776796) with the risk of hypertension, atherosclerosis, coronary heart disease (CHD), myocardial infarction development in Russian population. The study involved 121

people aged 55 to 79 years divided into age- and sex-matched groups according to anamnestic data. The informed consent was obtained from all patients. Genotyping of polymorphic locus was carried out by allele-specific real-time polymerase chain reaction method using commercial kits (Litech, Russia). Statistical data processing was performed using MS Excel and the online calculator (http://gen-exp.ru/calculator_or.php). Individuals with homozygous 4G/4G genotype have CHD development increased risk (OR = 2.67; 95% CI 1.11–6.42) and increased total cholesterol, triglycerides and atherogenity index. Despite some changes in the distribution of alleles and genotypes frequencies of the *SERPINE1* gene between the studied groups of patients diagnosed with hypertension, atherosclerosis, myocardial infarction and control statistically significant differences were not found. Thus, the investigated polymorphic 4G/4G genotype of *SERPINE1* gene is a reliable marker of CHD, whereas in the case of hypertension, atherosclerosis, myocardial infarction development the association was not found. The study was performed with the support of Russian science Foundation grant No. 15-15-10022.

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E-P05.27

The association of single nucleotide variations of the *HDAC9* (rs13246896), *CAMK2B* (rs35089892), *GACAT3* (rs62116755) with sudden cardiac death

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Introduction: Single nucleotide variations (SNVs) of the *HDAC9* (rs13246896), *CAMK2B* (rs35089892), *GACAT3* (rs62116755) were identified in own genome-wide association study (GWAS) as associated with sudden cardiac death (SCD). The aim of this work is confirm the association of rs13246896, rs35089892, rs62116755 with SCD in a case-control study. Materials and Methods: A sample of SCD cases (n = 383, mean age 53.3 ± 8.8 years, men - 70.9%, women - 29.1%) was formed using the WHO criteria; the control sample (n = 385, mean age 53.1 ± 8.3 years, men - 68.3%, women - 31.7%) was selected according to sex and age. DNA was isolated by phenol-chloroform extraction. The groups were genotyped for the

selected SNVs by RFLP-analysis according to original methods. The data were statistically processed using χ^2 test according to Pearson, two-sided Fisher's exact test with Yates' correction for continuity. Results: No statistically significant differences in the genotype and allelic frequencies of rs13246896 (*HDAC9*), rs62116755 (*GACAT3*) between SCD cases and control were detectable. Genotype TT of rs35089892 (*CAMK2B*) is associated with protective effect against SCD (p = 0.01, OR = 0.49, 95%CI 0.28–0.84). Conclusions: rs13246896 (*HDAC9*) and rs62116755 (*GACAT3*) are not associated with SCD. rs35089892 (*CAMK2B*) is associated with protective effect against SCD.

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Polymorphism - 634 G/C (rs 2010963) of VEGF-A gene in the development of hypertension in perimenopausal women

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One of the major endothelial factors stimulating angiogenesis is a vascular endothelial growth factor (VEGF). One of the possible mechanisms of increasing of the VEGF concentration in blood may be a genetic predisposition to an increased VEGF synthesis. The aim was to determine the effect of genetic polymorphism -634G/C (rs2010963) of the VEGF-A gene and formation of AH combined with obesity in premenopausal women. In study were 115 women with stage II of AH, aged 45 to 53 years in perimenopause included. According to menopausal status: 45 premenopausal and 50 menopausal women. The control group was consisted of 20 healthy premenopausal women. The VEGF concentration was determined by ELISA. The study of the allelic polymorphism -634 G/C (rs 2010963) VEGF-A gene was performed by polymerase chain reaction. The VEGF level was also significantly higher in women with the GG genotype (436,4[315,2; 772,8]) pg/ml comparing with the genotype CG (314,6[222,9; 449,4]) pg/ml and the genotype CC (261,8[127,5; 268,8]) pg/ml, there were any significant differences among women with the CG and CC genotypes in the premenopausal group. The VEGF level was significantly higher in the menopause group with the GG genotype (535,2[290,5; 726,8]) pg/ml comparing with the genotype CG (252,4[217,0; 363,8]) pg/ml and the genotype CC (226,9[197,9; 252,8]) pg/ml. The VEGF level was significantly higher among women with the genotype GG comparing to the CC. The relationship between the

level of VEGF and the carriage of GG genotype polymorphism -634G/C(rs 2010963) VEGF-A can be regarded as predictor of AH in perimenopausal women.

M. Iaresko: None.

E-P06 Metabolic and mitochondrial disorders

E-P06.01

Audiological and vestibular pathologies in subjects harbouring the mtDNA mutation A3243G

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Introduction: The mitochondrial DNA point mutation A3243G is known to express mainly two syndromes: Maternally Inherited Diabetes and Deafness (MIDD) and Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS). Research has found Sensorineural Hearing loss (SNHL) to be the most frequent symptom in subjects bearing the A3243G mutation. Dysfunction of the vestibular organs is, however, scarcely investigated. With this study, we aim to examine the impact the A3243G mutation has on the inner ear. This was done by performing extensive vestibular and audiological examinations.

Method: Eight subjects with blood verified A3243G mutation underwent thorough audiological and vestibular examinations: Tone and speech-audiometry, video Head Impulse Test (v-HIT) of all six semi-circular canals (SCC), ocular- and cervical-Vestibular Evoked Myogenic Potential (VEMP), and otoneurological examination. Subjective symptoms were evaluated with the Dizziness Handicap Inventory (DHI) questionnaire.

Results: SNHL was found in seven subjects, one suffered a conductive hearing loss, combined mean PTA₄ of 58,4 dB. Speech Discrimination (n = 7) ranged from 24% to 100%, mean at 74%. v-HIT (n = 42) found pathology in nine lateral SCCs, five posterior SCCs and one anterior

SCC, three measurements were inconclusive. All (n = 14) o-VEMPs were absent, nine c-VEMPs were absent and two were inconclusive. Through the DHI, six subjects reported none to mild dizziness, one reported moderate, and one severe dizziness.

Conclusion: Our population showed pathology from all the audiological and vestibular end organs. Our findings indicated that the pathology was situated within the end organs, and not within the superior- and/or inferior vestibular nor cochlear nerves. **Grants:** None

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E-P06.02

Recurrent disease-causing mutation in Hungarian patients with acute intermittent porphyria

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Introduction: Acute intermittent porphyria (AIP) is an autosomal dominantly inherited disorder, caused by reduced activity of the enzyme hydroxymethylbilane synthase (HMBS, also known as porphobilinogen deaminase), a specific enzyme in the heme biosynthetic pathway. Among diverse clinical manifestations, the most relevant features are life-threatening acute neurovisceral attacks with severe abdominal pain. Mutations of HMBS gene are in the background of clinical symptoms. Detection of mutations can confirm the diagnosis of AIP in situations where clinical data are inconclusive, and prevent at-risk relatives from acute attacks. **Materials and methods:** In the present study, we investigated 31 unrelated Hungarian families with AIP, using Sanger sequencing method. **Results:** Missense mutations were detected in 15/31 (48%) of investigated families. Beside them, 1/31 (3%) nonsense, 3/31 (10%) splice-site, 9/31 (30%) frameshift mutations were identified, while in three cases (10%) we could not find any alterations in the HMBS gene with Sanger sequencing. We observed six previously unpublished mutations. A previously not described small insertion (p.Ser75Lysfs*8; c.224insA; g.9354insA) were present in three, and a known missense mutation (p.Gly111Arg; c.331 G > A; g.9871 G > A) in 12/31 (38%) unrelated families. **Conclusions:** Similar findings of a single recurrent disease-causing mutation in Swedish and Argentinian population were already published. Interestingly, p.Gly111Arg mutation was also common (12/26

patients, 46%) in Argentina. Our findings further support the theory that founder mutations might be in the background of AIP, but -according to the high prevalence of a mutation in two distant populations- also suggest, that there might be some mutational hot-spot regions in the HMBS gene.

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E-P06.04

Follow up four cerebrotendinous xanthomatosis patients; importance of early diagnosis and treatment

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Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive disorder causing by the lack of sterol 27-hydroxylase enzyme that results in disabled of primary bile acid synthesis. CTX is caused by mutations in the *CYP27A1* gene that lead to the defect of the enzyme, but also to accumulate cholesterol and cholestanol in lipophilic tissues. Chronic infantile diarrhea probably the earliest clinical finding, bilateral juvenile cataracts secondary, preceding xanthomas in the tendons and progressive neurological disorder constitute the broad clinical spectrum of CTX. Here we present four patients from two unrelated families with mutation of the *CYP27A1* gene (homozygous c. C808T; p.R270X and homozygous c.C1333T; p.Q445X, respectively). The age range of all patients was from 5 years old to 17. At first family, two patients had chronic infantile diarrhea, bilateral cataract and mild intellectual disability. MRI findings showed cerebellar atrophy and T2 hyperintensity at dentate nucleus. In the other family, patients had chronic infantile diarrhea and mildly mental retardation but no xanthoma and cataract. Besides their MRIs were normal at initial examination. Only dysmorphic finding was coarse hair. Chenodeoxycholic acid (CDCA) treatment provided significantly neurological improvement in all specifically mild cases. Early diagnosis of CTX is crucial in order to prolong life expectancy but also to initiate early genetic counselling with parents concerning future pregnancies. Mildly symptoms should be taken into account for CTX patients in early diagnosis.

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E-P06.05

A newborn with combined oxidative phosphorylation deficiency 1 diagnosed by targeted next-generation sequencing

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Introduction: Combined oxidative phosphorylation deficiency is an autosomal recessive multisystem disorder with variable manifestations resulting from a defect in the mitochondrial OXPHOS system. Combined oxidative phosphorylation deficiency-1 (COXPD1) leading to early fatal progressive hepatoencephalopathy is caused by homozygous or compound heterozygous mutation in the gene encoding the mitochondrial elongation factor G1 (GFM1). Materials and Methods: We report a newborn boy with clinical presentation of severe treatment resistant metabolic acidosis and encephalopathy from the first hours of life leading to death in early neonatal age. The infant was born at term from 4th pregnancy (three successful previous pregnancies) of healthy parents, with ultrasound findings of hydronephrosis two weeks before birth. From 18th hour after birth the newborn was in deterioration with impaired consciousness and hypotonia, severe metabolic acidosis, hypoglycemia and significantly elevated plasma lactate levels. The tests of urine organic acids by GC/MS and blood aminoacids and acylcarnitines by MS/MS revealed generalized aminoaciduria (the highest deviation from the normal range seen in alanine), massive lactic aciduria, severe ketonuria, elevated excretion of 3-hydroxydicarboxylic acids and elevated branched-chain amino acids. Targeted next-generation sequencing using TruSight Inherited Disease panel (Illumina) was performed. Results: Two variants in heterozygous state were found in the GFM1 gene: a known pathogenic - c.748 C > T (p. Arg250Trp), and a likely pathogenic - c.1318 T > C (p. Ser440Pro), correlating with the observed clinical phenotype. The patient's mutations were confirmed by Sanger

sequencing. Conclusions: Targeted next-generation sequencing may help in diagnosis of rare metabolic diseases with genetic heterogeneity and relatively non-specific clinical phenotype in neonatal age.

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E-P06.06

Frequency of *CYP21A2* gene in a pediatric Portuguese population with congenital adrenal hyperplasia

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The most common cause of congenital adrenal hyperplasia (CAH) is 21-hydroxylase deficiency (21-OHD), which is caused by recessive mutations in *CYP21A2* gene. The clinical phenotype of the disease is classified as classic (saltwasting and simple virilizing forms) and as non-classic. The severity of the disease is described to be often correlated with the enzymatic activity. Genetic testing confirms the disease and is crucial for familial studies. The aim of this work is to do clinical and molecular characterization of the cohort of subjects observed at the Hospital Pediatrico de Coimbra (Portugal) with the clinical suspect of CAH. *CYP21A2* gene analysis was performed in 77 unrelated Portuguese patients (50 female, 27 males) with clinical and endocrinological suspicion of CAH, using direct *CYP21A2* sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis. *CYP21A2* mutations were identified in 71/77 (92%) of patients. In one of the six remaining patients, a homozygous mutation in *HSD3B2* gene, encoding the 3-beta-hydroxysteroid dehydrogenase enzyme, was found. 35.2% (25/71) of patients were homozygous; 60.5% (43/71) were compound heterozygous and in 4.2% of cases (3/71) only one heterozygous mutation *CYP21A2* mutations was identified. The most common point mutations were V281L and g.655 A/C>G that account for more than 50% of mutations observed in these population. All variants were described except a novel missense mutation identified in a salt-wasting patient, g.1173 T>C(p.Trp201Arg). Overall, our study provides a detailed clinical and molecular characterization of a large cohort of CAH Portuguese

patients. The overall concordance between observed phenotype and the genotype-predicted phenotype was 89%.

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E-P06.08

Disease-targeted sequencing:*CFTR* gene targeted exome sequencing in Turkish cystic fibrosis patients with a novel mutation

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Introduction: Cystic fibrosis (CF) is an autosomal recessive disorder that causes life-threatening conditions. Approximately 70,000 individuals are affected worldwide and especially those of European descent. Severely affected CF patients with positive sweat test were referred for *CFTR* targeted exome-sequencing. Methods: 125 potential CF patients have been sequenced using IonPGM sequencing technology and analyzed with Ion Reporter software. Genetic variants in *CFTR* gene known to cause CF were filtered. Clinically important variants were checked and confirmed by Sanger sequencing. Results: Disease-targeted *CFTR* exome analysis exposed (likely) pathogenic variants in 27.2% (n:34) of all cases. This study led to the identification of 26 different *CFTR* gene mutations, including 19 missense, 2 nonsense, 2 frameshift, and 3 splice site mutations. Of these, one was a novel missense variation at exon 17 A (p.Glu1044Gly; 3131 A>G) and the most common ones were p.Leu997Phe, c.2789 + 5 G>A, and p. Phe1052Val with a mutant allele frequency of 8.82%, 7.35% and 5.88%, respectively. Here, we report p. Glu1044Gly as a novel causative mutation for CF disease. POLYPHEN and SIFT analysis revealed that, this variation is predicted to be deleterious and damaging with a score of -4.80 and 0.002, respectively. Conclusion: As a single-gene disorder, CF is the most common autosomal recessive

disorder. Since the custom of consanguineous marriages in Turkey is still high (app 22%), the frequency of autosomal recessive diseases can be higher than expected. Using disease-targetted exome sequencing will be valuable to identify both known and new disease-causing variants of *CFTR* in a short time period.

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E-P06.09 **Mitochondrial respiratory chain association with DMT2 and obesity in spanish population**

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Introduction: DMT2 and Obesity are the main metabolic disorders worldwide. Mitochondrial respiratory chain (MRC) alteration can contribute to their development. Our objective is to analyze possible association between MRC genes and these diseases.

Materials and methods: 3731 subjects from 3 Spanish population based studies (Hortega, Pizarra and Segovia) were studied. 48 single nucleotide polymorphisms (SNPs) of chromosomal genes of the MRC were genotyped. The significantly associated genes with Obesity and DMT2 were sequenced in a part of the sample. Identified SNPs with different allelic frequency between cases and controls were genotyped at the whole population.

Results: Significant associations with DMT2 and obesity, were observed with SDHB, SDHD, NDUFS5 and

NDUFS1 genes in the studied sample. The sequencing studies identified over 170 polymorphisms in promoter and exonic regions (including intronic boundaries), and only 9 had different proportion between cases and controls. We have analyzed these polymorphisms in order to know if they could be responsible for the previously found associations. 2 of these polymorphisms were associated with T2D and glycemia, and 3 with obesity and BMI. We haven't seen any clearly functional polymorphism responsible for these associations, although in silico analysis indicate possible effects of these SNPs in splicing.

Conclusions: Some genes codifying MRC can be involved in BMI, obesity and DMT2, although functional studies must be performed to elucidate alterations involved and their functional effect.

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E-P06.10 **Novel mutations of the ETFDH gene in three Italian families with lipids storage myopathy**

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Multiple acyl-CoA dehydrogenase deficiency (MADD; MIM# 231680) is a rare autosomal recessive disorder often characterized by lipid storage myopathy (LMS). In most MADD patients the disease is caused by mutations in the ETFDH gene (MIM # 231675), encoding the ETF dehydrogenase. Patients are mainly affected by recurrent episodes of lethargy, vomiting, hypoglycemia, metabolic acidosis and hepatomegaly, often preceded by a metabolic stress. Sometimes, muscle involvement, myalgia, weakness, and LMS occur. MADD results in large excretion of glutaric, lactic, ethylmalonic, butyric, isobutyric, 2-methylbutyric and isovaleric acids; indeed the disease is also known as "glutaric aciduria type II". To our best knowledge, 136 different ETFDH mutations have been described in about four hundred MADD patients. Here we report the clinical and genetic findings of three Italian

families with four affected members. In our patients we identified three novel ETFDH missense mutations (p.L138F, p.T151A and p.L550P), whereas the remaining three (p.A187V, p.W343R and p.D511N) have already been reported. Our patients had a juvenile/adult onset form with generalized muscle weakness, low muscle carnitine, and lipid storage in muscle. A variable decrease of OX-PHOS complexes documented mitochondrial involvement. Muscle ultrastructural analysis showed massive increase of intracytoplasmic lipid droplets, which were usually localized nearby mitochondria and were found decreased after riboflavin supplementation. Although the diagnosis of multiple acyl-Coenzyme-A dehydrogenase deficiency in adult life is difficult, it is rewarding because of the possibility of treating patients with carnitine or riboflavin with full recovery.

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E-P06.11

Marked reduction in the excretion of ethylmalonic acid following liver transplantation in a patient with ethylmalonic encephalopathy

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Ethylmalonic encephalopathy is a rare autosomal recessive disorder due to mutations in the gene ETHE1 which encodes for a mitochondrial matrix protein involved in metabolism hydrogen sulfide and thiosulfate. The condition often presents in infancy with hypotonia, seizures, petechiae and acrocyanosis. Death usually occurs in early childhood. The biochemical findings of the disorder are largely secondary to the inhibition of cytochrome c oxidase and short chain fatty acid oxidation by hydrogen sulfide leading to the accumulation of lactic and ethylmalonic acids respectfully. Treatment has been largely supportive, however, treatment with oral metronidazole and N-acetylcysteine has shown some promise (Visconti, 2010). Recently, a single case report of a successfully liver transplant in a patient with ethylmalonic encephalopathy was published (Dionisi-Vici, 2016) giving hope that the condition may be amendable to transplantation. Here we present a second case of successful liver transplantation in an infant with ethylmalonic encephalopathy including; the clinical presentation and

characteristic biochemical findings. Following transplantation there was 10 fold reduction in urinary ethylmalonic acid excretion. We present data of pre and post-transplant metabolite excretion and postulate that the reduction in circulating metabolites is a surrogate marker for improved hydrogen sulfide detoxification.

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E-P06.12

exome sequencing detected the splice site mutation in the SERAC1 gene

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Exome sequencing has been and will be increasingly utilized in genetic determinants of various inherited diseases. In the present study we applied exome sequencing to a patient with symptoms including; brain atrophy, deafness, vomiting, jaundice, cholestasis, respiratory problems, motor delay, seizure, mental retardation, scoliosis, growth retardation, spasticity, muscle weakness & dysphagia. At result we found an insertion (chr6, 158571484, C > CCATG) in SERAC1 gene with homozygous genotype in the patient and heterozygous genotype in her unaffected parents (rs797045105). Mutations of SERAC1 cause of 3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like (MEGDEL) syndrome. Notably bioinformatics analysis using mutation taster (prob > 0.99) and DDIGin (prob = 86.51) showed this mutation is diseases causing. This variant had been reported in Clinvar database as likely pathogenic variant. The variant location is in the boundary of exon 4 and intron 4–5. Interestingly Sanger sequencing of SERAC1 cDNA confirmed the insertion of nucleotides in the spliced transcripts of the gene so it is predicted to be pathogenic due to the production a frame shift, Gly89fs. SERAC1 encodes for a protein with a serine-lipase domain. This variation disrupts the potential of SERAC1 mRNAs for coding this domain. These findings emphasize a significant of role of exome sequencing application as genetic test to identify and characterize the comprehensive spectrum of genetic variation and classification for the patients with metabolic disorders.

A. Sedaghat: None.

E-P06.13**Novel frameshift deletion in the *GLA* gene as a molecular cause of Fabry disease**

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Fabry disease is an X-linked multisystemic disorder characterized by lysosomal deposition of globotriaosylceramide (GL-3) in various cell types. Molecular causes of the disease are mutations in the gene *GLA*, which encodes the enzyme α -galactosidase (α -Gal A). The clinical picture is highly heterogeneous in terms of symptom presentation and severity. The diagnosis of Fabry disease should be considered in males and females with the signs of periodic crises of burning pain in hands and feet (acroparesthesias), cutaneous lesions of blood vessels (angiookeratomas), abnormalities of sweating capability (most often hypohidrosis), opacities of chornea and lens, early-onset stroke, left ventricular hypertrophy or renal insufficiency of unknown etiology. Considering late-onset variants of the disease prevalence is estimated as 1 in 3.000. Here we present a family with clinical presentation of Fabry disease in several individuals. *GLA* sequence analysis of the female index patient suffering from cardiac symptoms revealed the novel 4-bp deletion c.848_851delAGAT in heterozygous state. This variant is predicted to result in a frameshift leading to termination of protein synthesis 33 amino acids downstream of the variant and truncating the original protein length by 107 residues. The same variant was detected in the brother and the son of the index patient, both also being clinically and biochemically affected. To our knowledge, the detected mutation in the *GLA* gene has not been described before. We discuss clinical features and pathogenesis of α -galactosidase deficiency in the context of our molecular finding and provide evidence for cosegregation of the novel deletion and disease phenotype.

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E-P06.16***G6PD* Rare and Common Variants Analysis in Neonatal Hyperbilirubinemia in Indonesian Population**

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Neonatal Jaundice is a common finding in newborn babies in Asia, including Indonesia. In some cases the serum total bilirubin (STB) levels exceed the 95th percentile for hour of life (hyperbilirubinemia). Severe neonatal hyperbilirubinemia (NH) could lead to kernicterus and result in neonatal death. *G6PD* genetic variations have been reported to be associated with NH. This study aims to identify *G6PD* variants involved in the development of NH in the Indonesian population. We collected blood and DNA samples consecutively from 115 and 116 healthy term-neonates with and without hyperbilirubinemia. *G6PD* activity assay was measured with Randox method and NADP/NADPH ratio was measured using abcam kit. All exons and UTRs regions of *G6PD* gene were deep sequenced using MiSeq-Illumina. For statistical analyses, chi-squares test was used and p-value $p < 0,05$ was considered as significant. In this study *G6PD* deficiency was not significantly different between cases and controls (p -value = 0,470) and was not associated with the ratio of NADP/NADPH (p -value = 0,302). We identified approximately 340 variants in cases and controls, rare variants were identified more in cases (18%) than in controls (13.8%). Two and three novel missense rare variants were identified in cases (p.V369A and p.I167F) and controls (p.S160N, p.

M159V and p.I36T) respectively, none of those variants caused G6PD deficiency. The most frequent common variant identified in exon regions was p.Y437=. This variant was not associated with NH (OR = 0.8, 95%CI = 0.44–1.62, p-value = 0.60). Our study provides data of *G6PD* genetic variants identified in infants with hyperbilirubinemia in Indonesian population.

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E-P06.17

Clinical and biochemical peculiarities in patients with galactose-1-phosphate uridyl transferase deficiency, our experience in five patients

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The most frequent hereditary disorder of galactose metabolism is classic galactosemia and is caused by a deficiency in GALT (galactose-1-phosphate uridyl transferase). Patients diagnosed with this disorder show a potentially fatal phenotype after galactose intake in the first days of life. The exact mechanism(s) causing the complications and the time window in which the damage occur remain uncertain. The national newborn screening in our country is limited to PKU, congenital deafness and congenital hypothyroidism; this is why a fast analysis for urinary biomarkers in patients suspected for genetic metabolic disease is an emergency. We present the biochemical abnormalities, the clinical pictures and evolution in five newborn patients diagnosed with classic galactosemia after the first days of life; concentration of urinary galactose (between 9.120 and 12.700 mmol/molCreatinine) and galactitol (between 4.380 and 10.200 mmol/molCreatinine) were measured; the ¹H-NMR spectrum of urine helped in a fast diagnosis but also in comparing the biochemical peculiarities with clinical pictures in our patients.

Considering the differential diagnosis, we outline our results in an infant with hepatic failure that was suspected for a mild type of galactosemia. Metabolic profiling is an essential component that along with genetics, transcriptomics and proteomics data will permit a description of the interactions between metabolites. Our cases will contribute to a better understanding of biochemical phenotype and clinical effects. Beside, this report highlight the importance of an extension of New Born Screening in our country and the importance of differential diagnosis as fast as possible in the emergency department.

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E-P06.18

Classical galactosemia biochemical mimics by citrin deficiency

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Galactosemia is an autosomal recessive disorder of galactose metabolism, caused by a deficiency in the activity of the enzyme galactose-1-phosphate uridyl transferase (*GALT*), which is caused by mutations in *GALT* gene. The estimated annual incidence is 1 in 23 000–44 000 in the European population. We report data from a 5-day-old boy who was hospitalized in the Neonatology Clinic with severe indirect hyperbilirubinemia and poor weight gain. The child had been exclusively breastfed. The patient was treated with phototherapy, but continued to deteriorate. He has vomiting and losing weight. An examination and laboratory tests indicated increased level of liver transaminases, alkaline phosphatase and ammonia. His lab analysis worsened, and he was in metabolic acidosis. As far as Latvia do not have newborn screening for galactosemia and based on clinical suspicion for classic galactosemia the patient was sent for selective metabolic screening. The carbohydrates (TLC), amino acids (HPLC -UV) and organic acids (GC-MS) analysis were done. The results indicated two possible diagnoses: 1) classical galactosemia because of high urinary galactose concentration and generalized aminoaciduria and 2) citrin deficiency due to of high plasma citrulline, tyrosine, methionine, phenylalanine, and threonine concentrations. Galactose is also increased in some cases of citrin deficiency. A urinary organic acid profile was indicative for liver damage or disease. After administration of a galactose-free diet, which is recommended for both, citrin deficiency and galactosemia, patient's clinical status

normalized. The diagnosis of galactosemia was subsequently confirmed and the alternative diagnosis of citrin deficiency was excluded by molecular analysis.

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E-P06.19

The prevalence of Q188R mutation in Classical Galactosemia patients from Republic of Moldova

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Classical galactosemia is caused by a deficiency in activity of the galactose-1-phosphate uridylyl transferase enzyme (GALT; EC 2.7.7.10; OMIM 230400), that leads to severe life-threatening disease affecting the liver and/or eyes.

Material and methods: We report on 8 children with impaired galactose metabolism. The screening test for blood Galactose level, GALT activity and NMR spectroscopy of urine (Galactose, Galactitol) were used for diagnose and evaluation. Only patients having null GALT activity were investigated by PCR-RFLP assay that is used to identify the *Q188R* mutation of *GALT* gene.

Results: Three children with neonatal onset and null GALT activity manifested liver affection and sepsis (*E.coli*) after breastfeeding during the first week of life. A positive screening test and high urine Galactose [28.7–50.7 mol/molCrea] and Galactitol [\sim 10 mol/molCrea] were appreciated before starting free-lactose/galactose diet, after diet Galactose level was normalized in contrast to Galactitol concentrations [0.3–1.0 mol/molCrea]. In those patients with GALT deficiency the *Q188R* mutation has been found in 50% of cases. In one child with classical galactosemia *Q188R* mutation was identified in homozygous state and in another one in heterozygous compound form.

Conclusion: The obtained data revealed that *Q188R* mutation of *GALT* gene is associated in 50% of Moldavian classical galactosemia patients with null GALT activity, neonatal onset and high concentrations of specific metabolites in body fluids profiles.

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E-P06.20

Hurler syndrome study in Iran with reporting two novel mutations

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Introduction: Hurler syndrome is the most severe form of mucopolysaccharidoses which is a kind of lysosomal storage disorders. Hurler syndrome is one of the autosomal recessive inborn errors of metabolism with the incidence of 1:100000 in every live birth. Patients show different symptoms like cloudy cornea, scoliosis, hearing impairment, facial anomalies, Joint stiffness, liver and splenomegaly. This syndrome is caused by mutations in α -L-iduronidase (IDUA) gene which is located on 4p16.3 and has 15 exons encoding Iduronate Sulfatase. In this study we investigate IDUA gene mutation in 7 affected Iranian Families using short tandem repeat (STR) markers and Sanger sequencing. **Material and methods:** In the present study, mutations in IDUA gene were analyzed in a total of 7 Iranian families referred to Kawsar Human Genetic Research Center. Informed consent forms were obtained and DNA extraction was performed using salting out procedure. Haplotype analyses were done using STR markers. These 7 families showed segregation of the disease with the IDUA gene. Subsequently all exons and introns boundaries of the IDUA gene were sequenced by Sanger sequencing. Potential pathogenicity of the novel variants were evaluated by on line soft wares such as Fathmm, Polyphen-2, Hope. **Results:** We identified 3 missense mutations which 2 of them were novel. The mentioned soft wares all revealed that the novel mutations could be pathogenic ones. **Conclusion:** The obtained results can increase our understanding about etiology of Hurler syndrome in Iranian population and it is helpful in genetic counseling and genetic diagnosis of this disease In Iran.

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E-P06.22**Case report of hyperphenylalaninemia BH4A in the Rostov region of Rostov region, Russia**

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Hereditary hyperphenylalaninemias (HFA) are associated with a deficiency of tetrahydrobiopterin (BH4) - are genetically heterogeneous group of progressive neurological disorders, caused by mutations in genes which encode the enzymes involved in synthesis or regeneration of BH4. Results: Patient L.A. - baby 3,5 years old. In terms of the neonatal screening the child has been called to the geneticist due to increased neonatal levels of phenylalanine (FA) to 19,26 ml%. At the retest the FA - 29,24 ml%. Thanks to the special amino acid mixture without phenylalanine and low protein diet FA values decreased by 0,88 ml% - 2,35 ml%. The psychomotor development was in line with the age. In carrying out molecular genetic diagnostics in the PTS gene mutations detected, the genotype of the patient presented N52S/P87S, which made it possible to diagnose - HFA BH4 A. This condition refers to a progressive disease of the nervous system and has severe clinical manifestations, as well as the only possible treatment is the use of the drug saptoperin dihydrochloride (Kuvan). Positive dynamics was shown, and a sharp decline in the FA to 0.85% while taking the drug saptoperin dihydrochloride (Kuvan). Conclusions: This case shows that even with a positive effect on the ongoing diet therapy and the absence of mutations in PAH gene patients with hyperphenylalaninemia and PKU need for DNA diagnostics for genes for excluding atypical forms of PKU. This work was partially funded by RFBR (Russian Foundation for Basic Research) grants 17-04-00288, 15-04-01859 and RRF 17-15-01051(Russian Research Foundation).

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E-P06.23**A 7-month old female with MEGDEL syndrome presenting with transitory neonatal 3-methylglutaconic aciduria and challenging confirmation of the diagnosis**

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MEGDEL syndrome with 3-methylglutaconic aciduria (3-MGA) is characterized by dystonia, deafness and bilateral basal ganglia involvement (Leigh-like syndrome). It is caused by biallelic mutations in the *SERAC1* gene. We present a girl who was born at term with Apgar scores 3/4/4. Severe birth asphyxia was diagnosed (unconsciousness, muscular spasticity without active movements, multi-organ insufficiency) and therapeutic hypothermia was applied for 72 h. She had subclinical seizures and super-refractory status epilepticus. Sensorineural deafness and central hypothyreosis was additionally diagnosed. At 12 days, brain MRI showed asymmetric cerebral white matter T1 hypo- and T2 hyperintensity and decreased volume of basal ganglia and thalami compared to MRI on the 5th day. At 7-month the child has severe developmental delay, swallowing dysfunction, minimal activity and dystonias. Metabolic analyses showed twice slightly elevated 3-MGA in urine and increased serum lactate during first 17 days of the life. There were features of cataract and cardiomyopathy in the first weeks of life and due to that first *CLPB* gene defect was first excluded, but later these features disappeared. Trio whole exome sequencing revealed heterozygous previously reported pathogenic mutation c.1822_1828 + 10delinsAC-CAACAGG p.(Ser608Thrfs*5) (NM_032861.3) inherited from mother. Intensive reanalysis of *SERAC1* gene was performed only due to 3-MGA in urine. Read-depth analysis indicated possible paternally inherited deletion of the exon 5, which was later confirmed by MLPA and long-range PCR analysis. In conclusion, MEGDEL syndrome should be suspected in every new-born with Leigh-like

syndrome even with mild and/or transient increase of 3-MGA in urine. Work was supported by PUT355

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E-P06.24

Partial *ATP7A* gene duplication in Czech patient with Menkes disease

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Introduction: Menkes disease is a rare X-linked recessive disorder of copper metabolism caused by mutations in the *ATP7A* gene. The classical form is characterized by progressive neurological degeneration, connective tissue defects, tortuosity of blood vessels, abnormal kinky hair and is lethal in early childhood. More than 300 mutations have been reported in the *ATP7A* gene, but only 23 mutations are partial gene duplications. We report the first Czech patient with Menkes disease and duplication of several exons of the *ATP7A* gene. **Methods:** The *ATP7A* gene was initially screened for mutations by Sanger sequencing of all coding exons and flanking intronic regions. The copy number analysis was performed by MLPA using P104 SALSA MLPA probemix (MRC-Holland, NL). cDNA was prepared from RNA extracted from patient's blood and duplicated region was amplified using primers flanking the suspected duplication. PCR product was sequenced by Sanger method. **Results:** MLPA revealed a hemizygous *ATP7A* duplication comprising exons 5 to 10 and the duplication was confirmed by cDNA analysis. The same duplication was subsequently observed in a heterozygous state in patient's asymptomatic mother. She was offered prenatal genetic testing and the duplication was identified in a hemizygous state in chorionic villi sample. **Conclusion:** We confirmed the first Czech case of Menkes disease caused by partial *ATP7A* duplication. Our finding expands the spectrum of reported *ATP7A* duplications and emphasizes the importance of using various techniques to detect different types of pathogenic mutations. The study was supported by grants MZCR RVO-VFN64165/2012, UNCE 204011/2012, Progres Q26/LF1, GACR 14-36804G.

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E-P06.25

Combined effect of PPARGC1A - Gly482Ser and MTHFR - Ala222Val polymorphisms on the metabolic syndrome in a central Romanian population

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Introduction: PPARGC1A is a crucial transcription factor involved in the control of energy homeostasis, adapting metabolism to fasting, exercise or cold. MTHFR is a central enzyme of folate metabolism linked to methylation reactions and epigenetic control. Methyl donor deficiency has been suggested to produce detrimental effects on fatty acid oxidation and energy metabolism by impaired PPARGC1A methylation. Combined effect of their common polymorphisms rs8192678 (Gly482Ser) and rs1801133 (Ala222Val-C677T) was explored in a case-control study on a Romanian population. **Material and methods:** 196 metabolic syndrome patients and 102 control subjects have been investigated metabolically and by PCR-RFLP (5'-CAAGTCCTCCAGTCCTCAC-3'/5'-GGGGTCTTGA-GAAAATAAGG-3', MspI; 5'-CATCCATTG-CAGCTTAC-3'/5'-GACGGTGCGGTGAGAGTG-3', Hinfl). **Results:** In the affected and control group, minor allele frequencies of rs8192678/rs1801133 were 33.16/30.35 vs 26.47/31.86%. While wild type allele combination decreased in patients, and the number of variant carriers increased, association of the suspected predisposing alleles remained similar (21.93 vs 35.29 and 28.06 vs 27.45%), and no increased risk for disease development could be demonstrated (OR = 1.64, p = 0.14; CI = 0.87-3.1). Metabolic parameters according to the four genotype-combinations showed no statistically significant differences in either group, though a worsening trend could be noted. In conclusion, common variant association of the presumably interdependent key regulators PPARGC1A and MTHFR involved in short- and long-term gene expression control could associate with supplemental metabolic profile impairment. Joint effects need to be addressed by finding adequate approaches to study gene interactions, in particular for rs8192678/rs1801133, motivated by allele frequency, role in early development of adult onset diseases and modifiable nature of deleterious effect by lifestyle interventions.

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E-P06.27**NMR Spectroscopy in diagnosis of several inborn errors of metabolism: methylmalonic acidurias, ketolysis defect**

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The emerging field of metabolomics, in which a large number of small-molecule metabolites are detected quantitatively in a single step, promises immense potential for early-diagnosis, monitoring, and understanding the pathogenesis of many diseases. The clinical/ biochemical findings in some inborn errors of metabolism (IEM) are often non-specific; an early differential diagnosis made in a single urinary sample it gives an important advantage. We present the spectrum of metabolites of urine from two patients with methylmalonic acidurias [a classical form for a newborn with a severe lethal acidosis, and the second child (3 years-old boy) having an intermittent form, presenting a hemophagocytic syndrome, being first interpreted as a myeloproliferative disease]. The fast results gave by urinary NMR-spectrum (Bruker Avance 400 MHz-Spectrometer) showing a high concentration of methylmalonic acid indicates the MMA diagnosis. Beside this, we present our results and the utility of this method for rapid diagnosis and follow up in a ketolysis defect (with very high 3-hydroxybutyric aciduria) identified in a 4 years old girl with two acute episodes of hypoglycemia during fasting due to intercurrent viral infections. The level of excretion of the metabolites in these three IEM has been well within the range of NMR detection. In the critical care setting, IEM that were not diagnosed through the neonatal screening should be considered as cause of acute neurologic, hepatic/ renal decline, rapid diagnosis being essential. We demonstrate the effective use of NMR-spectroscopic-profiles of urine in differential diagnosis in emergency situations, and the possibility of follow up, as well.

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E-P06.28**Comparison of mitochondrial DNA haplogroups in Parkinson disease, epilepsy patients and control individuals from Slovak population reveals T1 and I haplogroup association with increased risk**

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Mitochondrial dysfunction lies at the nexus of a wide range of metabolic and degenerative diseases, cancer, and aging. Unique quantitative genetics of the maternally inherited mitochondrial DNA (mtDNA) and major clinical consequences of even a subtle bioenergetic alterations are outlining their very complex etiology. Unsurprisingly, since mitochondrial dysfunction and/or complex I deficiency has been implicated in pathogenesis of Parkinson's disease (PD), consistent with their functional importance, certain mtDNA haplogroups and SNPs have been correlated with predisposition to various metabolic and degenerative diseases, cancers, and longevity. Not only mitochondrial function might be perturbed by mtDNA variation, either by recent deleterious mutations or ancient polymorphisms (haplogroups), also accommodation of somatic mutations and mtDNA copy number per cell are crucial as well. In order to shed more light on the role of inherited mtDNA variation in PD and epilepsy we have analyzed mtDNA variability in Slovak PD (228) and epilepsy (107) patients and control group from Slovak population (759). We found evidence for significant association of mtDNA haplogroup T1 with increased risk for PD and haplogroup I with increased risk for epilepsy. However, no or weak association have been found for major mtDNA lineages or previously described risk modifying lineages. Thus, the newly associated T1 and I haplogroups, might represent minor but serious genetic factors increasing the risk of PD and epilepsy, respectively. Sequencing of whole mtDNA molecules of patients' samples belonging to T1 haplogroup

identified coding region SNPs, which might be considered as possible target for future association studies.

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E-P06.29

Prenatal diagnosis of mitochondrial respiratory chain disorders caused by nuclear gene mutations

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[Background] Mitochondrial respiratory chain disorders (MRCDS) are the most common inherited group of metabolic disorders and are caused by sporadic or inherited mutations in either mitochondrial DNA (mtDNA) or nuclear DNA (nDNA). The incidence of this disease is > 1 in 5000 live births; in the pediatric population, about 25% of mitochondrial disorders are due to abnormalities in mtDNA, whereas the rest are due to defects in nDNA. **[Materials & Methods]** Our team used comprehensive genomic analysis to diagnose 149 patients (Kohda *et al.* PLOS Gen 2016). Of those patients, eight families (nine cases), with children who were diagnosed with mitochondrial disease caused by autosomal recessive mutations, requested prenatal diagnosis. After genetic counselling, six families (seven cases) decided to undergo prenatal diagnosis, of which four patients underwent chorionic villus sampling and three underwent amniotic fluid sampling. As contamination was suspected in one case who had undergone chorionic villus sampling, we performed additional amniotic fluid sampling. Two cases miscarried. **[Results]** Four cases diagnosed as heterozygotes continued the pregnancy and delivered healthy babies. Two cases were diagnosed as compound heterozygotes and hemizygotes, and their families chose induced abortion. **[Conclusions]** Severe phenotypes of mitochondrial disorders are prevalent, particularly in the

neonatal and infantile periods. These patients may be candidates for prenatal diagnosis with careful parental genetic counselling.

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E-P06.30

Novel mutation in the HNF1a gene in a family with MODY3

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Maturity-onset diabetes of the young (MODY) is a subgroup of diabetes mellitus (DM) characterized by an autosomal dominant inheritance and early onset. MODY 3 is a clinically progressive phenotype with severe hyperglycemia, glycosuria, and decreased renal function or microvascular complications, which can be reduced substantially by early treatment. Patients diagnosed with MODY 3 are at risk for diabetes associated complications. The causative gene of MODY3 is the hepatocyte nuclear factor (*HNF*)-1 *alpha* gene which plays an important role in the regulation of insulin secretion. The identification of the distinct molecular genetic alterations that are the underlying cause of the specific forms of DM is an important prerequisite to optimize the individual treatment of the affected persons. We describe a novel mutation in the *HNF1α* gene in a family with MODY3. The index was diagnosed at the age of 12 years. Molecular genetic analyses revealed in exon 4 of the *HNF1α* gene the nucleotide change c.775 G > C in a heterozygote state. The amino acid change from Valin to Leucin at amino acid position 259 is predicted to affect function of *HNF1α* protein. To our knowledge, this mutation in the *HNF1α* gene has not been described before. We report on family history, clinical and molecular genetic results of the family members. Clinical features presented by the patients of the family are discussed within the context of molecular results.

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E-P06.31

In silico analysis of novel mutations in BCKDHB gene in maple syrup urine disease patients from Iran

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Maple Syrup Urine Disease (MSUD) is a rare autosomal recessive disorder of branched-chain amino acid (BCAA) metabolism. The disease is mainly caused by mutations either in the BCKDHA, BCKDHB, DBT or DLD genes encoding components of the E1 α , E1 β , E2 and E3 subunits of branched-chain α -keto acid dehydrogenase complex (BCKDC), respectively. BCKDC is a mitochondrial enzyme which is responsible for the normal breakdown of BCAA. The rate of consanguineous marriage in Iran is 38.6%, so the prevalence of autosomal recessive disorders is higher in comparison to other countries. Consanguinity increases the chance of the presence of pathogenic mutations in a homoallellic state. This phenomenon has made homozygosity mapping a powerful tool for finding the probable causative gene in heterogeneous disorders like IEM (Inborn Errors of Metabolism). In this study, two sets of multiplex polymorphic STR (Short Tandem Repeat) markers linked to the above-mentioned genes were selected to identify the probable pathogenic gene. Families who showed a homozygous haplotype for the STR markers of the BCKDHB gene were subsequently sequenced. Four novel mutations including c.633 + 1 G > A, c.988 G > A, c.833_834insCAC, and a homozygous deletion of whole

exon 3 c. (274 + 1_275-1)_(343 + 1_344-1), as well as one recently reported (c. 508 G > T) mutation have been identified. Three families shared a common haplotype structure along with the c. 508 G > T mutation. Four other families revealed another similar haplotype with c.988 G > A mutation. Founder effect can be a suggestive mechanism for the disease. Additionally, structural models of MSUD mutations have been performed to predict the pathogenesis of the newly identified variants.

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MtDNA sequence analysis in the group of Lithuanian patients with clinically suspected mitochondrial disease

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Introduction: Mitochondrial disorders are clinically and genetically heterogeneous group of diseases. A correct diagnosis is challenging mainly due to the complexity of the clinical presentations and lack of classical diagnostic clues. In addition, these diseases are attributed to the mutations in the 16.6 kb mitochondrial genome and approximately 1500 genes encoded in the nuclear genome. This study was conducted to investigate possible disease-related mutations in mtDNA based on the clinical phenotype and family history of patients with suspected mitochondrial disease. **Patients and Methods:** In 2015–2016, 37 blood DNA samples of Lithuanian patients with clinically suspected mitochondrial disease (27 children, 10 adults) were studied. The Sanger sequencing of mtDNA was performed for all patients. All mtDNA variants identified were compared to the Mitomap, mtDP, mtSNP and other databases. **Results:** One patient was identified with pathogenic heteroplasmic mutation m.12147 G > A in *MT-TH* gene. Three rare mtDNA variants m.14687 A > G, m.4336 T > C, m.10044 A > G associated with mitochondrial diseases were identified in three patients. The vast majority of other variants were considered to be polymorphisms. MtDNA haplogroup analysis revealed a frequency of 62.5% of H haplogroup in the studied patients group. **Conclusions:** We obtained high-depth whole mitochondrial genome sequences to determine mtDNA variants associated with the development and

progression of mitochondrial disease. Rare mtDNA variants were found in 10.81% of the patients with suspected mitochondrial disorders. Supported by grant No TAP LLT-02/2015

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A case of a rapidly progressive form of Mucopolysaccharidosis type VI with a late diagnosis

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Introduction: Mucopolysaccharidosis type VI (MPS6; MIM #253200) is an autosomal recessive lysosomal storage disorder with progressive multisystem involvement, resulting from deficiency of arylsulfatase B (ASB). MPS6 shows a wide spectrum of symptoms from slowly to rapidly progressing forms, with short stature, dysostosis multiplex and degenerative joint disease, hepatosplenomegaly, corneal clouding, cardiac valve disease, hearing loss, and facial dysmorphism. Intelligence is generally normal. Methods: the authors present the clinical description of a patient with the diagnosis of MPS6 at 8 years of age, characterizing and comparing it with the main clinical features described in the literature. Results: Female patient firstly observed at 8 years old after being evacuated from Guinea-Bissau for clinical evaluation due to exophthalmia and short stature. Physical examination showed short stature, low weight, short neck, exophthalmia, macroglossia, ptosis of the tongue, umbilical hernia, short fingers, wide gait and metatarsus adductus. Corneal clouding and mitral and aortic regurgitation were described after ophthalmologic and cardiological evaluations. Audiometric evaluation revealed bilateral conductive hearing loss. Skeletal radiography showed dysostosis multiplex. Brain MRI revealed multifocal white matter alterations, dysmorphism of cervical vertebrae with C1-C2 instability, crano-vertebral junction stenosis and medullary compression suggesting MPS. Increased urinary levels of glycosaminoglycans and ASB deficiency were observed after analytical workout. Molecular analysis of ARSB gene confirmed the diagnosis of MPS6. Enzymatic replacement therapy was established. Conclusion: the late diagnosis in this patient due to lack of specialized medical care in her home country shows us the impact of early enzymatic replacement therapy in the prognosis of MPS6.

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E-P06.34

Perinatal death associated with mutations in ATAD3A gene in Polish family - new mitochondrial disease?

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The ATAD3A gene codes for mitochondrial membrane protein engaged in nucleoid organization, protein translation, cell growth, and cholesterol metabolism. As reported in October 2016, mutations in ATAD3A gene lead to neurodevelopmental disorders of diverse severity, with a core phenotype of global developmental delay, hypotonia, optic atrophy, axonal neuropathy, and hypertrophic cardiomyopathy. One of 8 patients reported, who harbored biallelic deletion of ATAD3A gene, died on day 13. We present a Polish family in which two children were born with the severe clinical symptoms including respiratory failure, myocarditis, pulmonary edema, encephalitis, seizures, multiple organ failures. The metabolic diseases were excluded after tandem mass spectrometry. Children died 13 and 7 days after birth. Using NGS and Sanger sequencing we have identified heterozygous point mutation in ATAD3A gene: p.Glu217Lys (rs756429611) and heterozygous deletion of exons 1–11. Unaffected parents were heterozygous for one of the ATAD3A mutations; the transmission pattern in this family was consistent with autosomal recessive inheritance. Additionally, father was also asymptomatic carrier for other rare ATAD3A mutation: p.Lys568Met (rs41285840). None of the affected children inherited this variant. The future study for the other members of the family and functional study will be continued.

E. Witkowska: A. Employment (full or part-time); Modest; Medgen. **A. Sobczyńska-Tomaszewska:** A. Employment (full or part-time); Modest; MedGen Medical Center. **K. Czerska:** A. Employment (full or part-time); Modest; MedGen Medical Center. **M. Kacprzak:** A. Employment (full or part-time); Modest; MedGen Medical Center. **A. Kostyk:** A. Employment (full or part-time); Modest; Kostyk i Kruczek Medical Center. **M. Korostynski:** A. Employment (full or part-time); Modest;

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E-P06.36

Inborn errors of metabolism: A common cause of non-immune hydrops fetalis in an Arab Omani population

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Introduction: Inborn errors of metabolism (IEM) are a rare cause of non-immune hydrops fetalis (NIHF) and seldom investigated for during routine diagnostic work-up. Considering the preference for consanguinity and increased incidence of autosomal recessive disorders in Oman, IEM may be contributing to a significant proportion of cases with NIHF. The purpose of this study was to calculate the incidence of NIHF in the Arab Omani population and to evaluate the diagnostic yield of a locally developed protocol to identify the etiology of NIHF in affected pregnancies. Methods Following systematic implementation of a locally modified diagnostic approach including molecular genetic testing and metabolic work-up of lysosomal storage disorders, a retrospective review was carried out to calculate the incidence of NIHF, and to assess the diagnostic yield of the protocol. Results An etiological diagnosis was established in 9 of 12 cases of NIHF (75%). The majority of cases (7/9) were caused by IEM which included a novel homozygous variant in the *AARS2* gene (5/7). The incidence of NIHF in our series was 1 in 270. Conclusion Incidence of NIHF is markedly increased as compared to published studies. IEM contribute to NIHF with a frequency of 58% as compared to the previously reported incidence of 1–18.5%. The *AARS2* variant accounts for a significant number of cases of NIHF in Oman.

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E-P06.37

Validation of genetic risk score predicting obesity in adults: Preliminary results

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Background: A genetic risk score (GRS) comprising 6 polymorphisms, located in genes *SH2B1*, *ETV5*, *SEC16B*, *TFAP2B* and *FTO*, was previously validated predicting individuals with high susceptibility to childhood obesity among Spanish population.¹ The objective of this study was to determine whether this GRS can predict obesity in a sample of Spanish adults.

Methods: This is a preliminary case-control study including a group of 73 morbidly obese adults (mean age 39 year-old), recruited from endocrinology service at General Hospital Valencia (Spain), between May 2016 and January 2017. A total of 138 normal weight adults as control group were also included. Genotyping was performed from whole blood samples by allelic discrimination Taqman assay. A multivariate analysis using logistic regression and adjusted by age and sex was used.

Results: The *SEC16B* rs3748792 polymorphism was nominally associated with body mass index (BMI) ($p = 0.007$), weight ($p = 0.009$) and body fat ($p = 5.0 \times 10^{-5}$). Nominal association was also found for the *ETV5* rs7634510 with BMI ($p = 0.04$) and weight ($p = 0.04$), and for the *FTO* rs17817449 polymorphism with BMI ($p = 0.03$) and weight ($p = 0.04$). Statistically significant predictor of obesity was also found for these 3 polymorphisms ($p < 0.05$). For the other 3 polymorphisms (*SH2B1* rs8055982, *TFAP2B* rs760900 and *FTO* rs9921255) no significant differences were observed.

Conclusion: This first preliminary analysis provides evidence for potential differential relative effects of a GRS for obesity between children and adults. The clinical significance of these results for implementation as part of weight management interventions needs further investigation.

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E-P06.38

Novel splice site mutations in Iranian Phenylketonuria patients

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Phenylketonuria (PKU) as an autosomal recessive disease is one of the most common inborn error of metabolism in our population. PKU frequency in Iran has been estimated to be about one per 5000 live births. Untreated patients show different levels of mental and physical retardation, so early diagnosis is very crucial in patient management. PAH encodes phenylalanine hydroxylase enzyme which converts phenylalanine to tyrosine, the essential neurotransmitter precursor. Any malfunction may lead to disease state. This study aimed to identify the causative mutations in PKU patients referred to Dr.Zeinali's Medical Genetics laboratory for mutation detection. Following DNA extraction, mutation screening of PAH gene was performed using direct sequencing of all exons and exon-intron boundaries of the gene. Different types of mutations were identified which include novel splice site mutations. The mutations were including two cases with c.169-1 G>A in intron 2, four cases c.510- 1 G>A in intron 5, two c.970-1 G>T in intron 9 and two c.1066-2 A>G in intron10. Heterozygosity of the identified variants was confirmed in the parents. Any changes in invariant GT AT sequence in donor and acceptor splicing sites always will disturb recognition splice site by spliceosome. This phenomenon leads to skipping an exon or retaining intronic sequence in mature mRNA in most cases. The Human Splicing Finder website, confirmed their crucial effect of these changes on the final splicing of the protein; these

alterations may lead to the enzyme dysfunction. So they are potentially pathogenic mutations and helpful in PGD (Pre-implantation Genetic Diagnosis) and PND (Prenatal Diagnosis) purposes

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E-P06.39

Novel PRPS1 mutation in a family with congenital hyperuricemia

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Superactivity of phosphoribosyl[Unsupported Character - Codename]pyrophosphate synthetase I (PRPPS) is a rare inborn error of purine metabolism that is characterized by increased levels of uric acid in blood and urine (OMIM 300661). The disorder is caused by gain-of-function mutations in the X-chromosomal gene *PRPS1*. In male patients, disease manifestation is in early childhood. Additional clinical characteristics include intellectual disability, hypotonia, and hearing loss. Heterozygous female mutation carriers have a later age of onset and a less severe clinical course. Only eight families with *PRPS1* gain-of-function mutations have been reported to date. We report on a 7-year-old boy with congenital hyperuricemia, urolithiasis, developmental delay, short stature, hypospadias and facial dysmorphisms. His mother also had hyperuricemia diagnosed at age 17 years but was otherwise healthy. A novel *PRPS1* missense mutation (c.573 G>C, p.Leu191Phe) was detected in the proband and his mother. Enzyme activity analyses confirmed superactivity of PRPP synthetase. The family reported here broadens the clinical spectrum of PRPPS superactivity and indicates that this rare metabolic disorder is associated with a recognizable facial gestalt.

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E-P06.40**A girl with two mutations in PIGN**

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The second daughter of healthy, Dutch parents presented with an extra nipple and distal hypoplasia of some digits. At the age of 4 months, she suddenly developed partial complex seizures and, on the same day, secondary generalized seizures with tonic contractions and apnea. With treatment she became seizure free. An electroencephalography showed a normal background pattern, multifocal epileptiform activity and no electroencephalographic seizures. EEG's in follow deteriorated and will be reported. Development is very delayed. Facial dysmorphism will be shown. An MRI at the age of 13-month showed normal anatomy and normal myelination. MR spectroscopy was normal as well. Metabolic testing in blood and urine was normal as well as flow cytometry. We report 2 years of follow up in her: she developed West syndrome and later localised epilepsy. Vitamin B6 (pyridoxine) has been reported to be beneficial in some GPI deficient patients; apart from B6 she has mult antiepileptic treatment. Family: the first daughter had benigne West syndrome and develops normal. The maternal sister died from epilepsy. Whole exome sequencing (targeted gene panels for Intellectual Disability and epilepsy) revealed compound heterozygous PIGN (phosphatidylinositol glycan class N, OMIM: 606097) variants; a paternally inherited p.Arg785His substitution and a maternally inherited p.Tyr249Cys substitution. *PIGN* is one of the genes involved in the glycosylphosphatidylinositol (GPI)-anchor biosynthesis and remodeling pathway. PIGN mutations have been described as causal for 'multiple congenital anomalies-hypotonia-seizures syndroom 1' (MCAHS1, OMIM: 61408).

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E-P06.42**Role of TCF7L2 gene polymorphism rs7903146 in Bulgarian obese adults in the development of prediabetes**

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The frequency of prediabetes is increasing rapidly worldwide. It is defined as an intermediate metabolic state characterized by impaired glucose tolerance (IGT) and fasting glucose, thus increasing the risk of developing type 2 diabetes mellitus (T2DM). However, only 50% of individuals with IGT progress to diabetes over their lifetime and it is still unknown whether previously identified diabetes risk genes can also determine risk for prediabetes. Several genetic studies associate polymorphisms in transcription factor 7-like2 gene (TCF7L2) with T2DM in adults, but its role for the development of prediabetes is still unclear. The aim of our study is to test if risk genotypes TC and TT of rs7903146 are more common in obese adults (BMI > 25) with increased homeostasis model assessment insulin resistance index (HOMA-IR) compared to obese controls with normal HOMA-IR. Analysis was done on a total of 218 patients, 122 of them with impaired HOMA-IR. DNA samples were analyzed by PCR - direct sequencing. Genotype and allelic distributions in patients presenting increased HOMA-IR (TT: 13%, CT: 38%, CC: 49%) and in patients with normal HOMA-IR (TT: 7%, CT: 47%, CC: 46%) provided small, but no significant difference ($p > 0.5$) between the two groups due to the higher percentage of TT homozygous in the group with increased HOMA-IR index. Our conclusion is that *TCF7L2* could be one of multiple susceptibility genes for the development of impaired glucose tolerance and prediabetes in obese adults. The study has been financed by a research grant B02/10/12.12.2014 from the Ministry of Education and Science, Bulgaria.

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E-P06.43

Positive association of C16069T, T16519C, T16362C, T195C and T152C variants in D-loop of mitochondria with recurrent pregnancy loss in Iranian Persian ethnicity

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Recurrent pregnancy loss (RPL) is traditionally defined as three or more consecutive pregnancy losses before 20 weeks of gestation. Genetic and non-genetic factors have been found to be associated with RPL. A few studies have been performed to find the association between RPL and mitochondria. The identification of the genetic variants that confer risk for RPL is essential for the detection of individuals at high risk. This study investigated the association of variations of D-loop with RPL. 24 cases and 110 female controls from Fars ethnicity were selected. Following gDNA extraction from blood, Genotyping were performed using direct sequencing of HV1&HV2 regions. Results were analyzed using SPSS. The comparison of allele and genotype frequencies between cases and controls by Chi-square test revealed the T, C and C variants confer risk for the studied individual in C16069T (P-value < 0), T16362C (P-value < 0.003) and T195C(P-value < 0.009) variants respectively. Our analysis showed C variant (T16519C, and T152C) has protective role and can reduce the risk of RPL (P- value < 0.001, P-value < 0.01 respectively) Our findings is consistent with a report from the study of the three variants (T16519C, T152C and T195C) studied by Seyyed Hassani et al. According to the high mutation rate in D-loop and a regulatory role in this region, it seems that mutations in this region can disrupt cell survival. Therefore, these variants can be recommended as an additional factor for determining the risk of susceptibility to RPL.

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E-P06.44

S-adenosylhomocysteine hydrolase deficiency: a Turkish girl with novel mutations in the AHYC gene

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S-adenosylhomocysteine hydrolase, encoded by AHCY gene on chromosome 20q11, catalyzes the division of S-adenosylhomocysteine to adenosine and homocysteine. It plays a key role in the transsulfuration-transmethylation cycle that regulates tissue methionine supply and distributes methyl groups among scores of substrates. Here we describe a girl with S-adenosylhomocysteine hydrolase deficiency that was the known tenth reported patient who died at age three months. The female proband was born at term by cesarean section and at birth, she was profoundly hypotonic and required intubation. The antenatal period of pregnancy complicated by polyhydramnios and fetal neurological sequellae history. The proband was the product of third pregnancy of a non-consanguineous parents. The first pregnancy was a boy with anencephaly who was aborted in the first trimester while the second was born at term with severe hypotonia and female newborn died at 4.5 months due to same symptoms. The MRI images of the proband showed hypoplastic cerebral hemispheres, vermis and peduncle. She had microcephaly and type4 lissencephaly. Cardiac ultrasonography revealed secundum atrial septum defect. Laboratory testing revealed elevated AFP, ferritin and prothrombine time and also severe hypoalbuminemia was detected. Physical examination was notable for synophrys, ear malformations, high arched palate and extensive oedema in whole body. The exome sequencing revealed compound heterozygosity for mutations in AHYC gene (p.T57I (c. 170 C > T)/p.V217M (c.649 G > A)) of the proband. The mother was a carrier for p.T57I(c. 170 C > T) and father was p. V217M (c.649 G > A). These mutations was not reported before but they were predicted pathogenic by in silico prediction analysis.

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E-P06.45

Heterozygous mutation in SCO1 gene cause multilocular cystic encephalopathy, leukodystrophy, lactic acidosis, epilepsy

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BACKGROUND & AIMS: The role of heterozygous mutations in SCO1 gene (SCO1: pThr 197 Ile) is not fully clarified. This novel variant has not been previously described in patients nor in controls. It is not listed in the NHLBI Exome variant database.

METHODS: Clinical methods, neurological examination, MRT of brain, next-generation sequence analysis of the coding exons and intron-exon boundaries of 139 mitochondrial nuclear genes, next-generation sequence analysis to screen for the common ISCU:c.418 + 382 G > C variant, array CGH using the current version of the Mito exon array to screen for deletions and duplications. The multiple oligonucleotide probes representing most exons and/or intronic regions in 128 of the genes in the mitochondrial nuclear gene panel to screen for deletions and duplications, PCR-SBT method of mtDNA.

RESULTS: The child present with intrauterine growth retardation, transitory neonatal hypoglycemia, ventricular septal defect, multilocular cystic encephalopathy, leukodystrophy, lactic acidosis, left hemiparesis, epilepsy, metabolic decompensation associated with infections. A heterozygous SCO1:c.590 C > T variant with unknown significance was identified in exon 4 of the SCO1 gene (ref seq NM_004589.2). A heterozygous deletion of 3 nucleotides C8ORF38:c.557_559delTTT with unknown significance (VUS) was identified in exon 5 of the C8ORF38 gene (ref seq NM_152416.2). PCR-SBT method showed 2 polymorphisms in the mtDNA: T6071C, A8512C. The treatment include oxcarbazepine, vitamins of group B, Leaton, Duocal, L-carnitine, Resveratrol. The seizures are well controlled with medication.

CONCLUSIONS: The authors show that the heterozygous mutations in SCO1 in association with heterozygous deletion of 3 nucleotides of C8ORF38:c.557_559delTTT cause MELAS in patient.

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E-P06.47

Trimethylaminuria, is mutational analysis of clinical utility? Experience from an Irish metabolic clinic

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Primary Trimethylaminuria (TMAuria) (OMIM 136132), is an autosomal recessive rare disorder which results in diminished capacity to oxidise the dietary derived amine trimethylamine (TMA) to its odourless metabolite Trimethylamine N-oxide (TMA-N-O). Severe Primary TMAuria has been classified as the percentage of unmetabolized free TMA in urine being > 40% and mild/moderate TMAuria- range: 10–39%. More than 30 variants of the Flavin monooxygenase 3 (*FMO3*) gene have been reported to cause Primary TMAuria. *FMO3* mutation analysis is often requested for family genetic counselling. We performed gene sequencing of the entire *FMO3* gene coding region for 6 Irish adult probands (Group A) with confirmed moderate to severe TMAuria (% TMA range 27–45) and for 3 adults with mild TMAuria (%TMA range 18–20) (Group B) that presented to the Irish adult metabolic centre over the last five years. We identified *FMO3* causative (loss of function) mutations in only 2/6 probands in Group A with no other *FMO3* common polymorphisms detected. One individual was compound heterozygous for the common mutation p.(Pro153Leu), c.458 C > T and a presumed novel mutation p.(Gly228Ser), c.682 G > A and the second proband was compound heterozygous also for p.(Pro153Leu) and another presumed novel mutation p.(Asp232Tyr), c.695 G > T. Two of the 3 individuals in Group B were homozygous for the *FMO3* common variant haplotype p. [(Glu158Lys;Glu308Gly)]. These results suggest that severe TMAuria is rare in Ireland and that *FMO3* mutation analysis may not be clinically indicated (and should not replace standard biochemical testing), unless there is a severe biochemical phenotype (free TMA urine levels > 40%).

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E-P06.50

Evaluation of T241M Polymorphism of DNA repair gene XRCC3 and susceptibility to Type 2 Diabetes

Mellitus and Diabetic Nephropathy in Turkish population

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Introduction: Increasing number of experimental and clinical studies suggests that there is a close link between hyperglycemia, oxidative stress, DNA damage and diabetic nephropathy. The aim of this study was to explore whether the T241M polymorphic variant of *XRCC3* gene is associated with an increased susceptibility to type 2 diabetes mellitus (T2DM) and diabetic nephropathy (DN) in Turkish population. Materials and Methods: The study population included 238 unrelated subjects residing in Istanbul, Turkey; 116 had type 2 diabetes mellitus, 50 had diabetic nephropathy and 72 had normal glucose metabolism. Polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) was used to determine the distribution of genotypes and frequency of alleles of T241M polymorphism of the *XRCC3* gene (rs861539). Results: There is no statistically significant association between T241M polymorphism of *XRCC3* gene and T2DM or DN (OR 0.121, CI 0.89–2.73 and OR 0.588 CI 0.43–1.67, respectively). The multiple logistic regression revealed that being male ($p < 0.05$), older than 45 years of age ($p < 0.001$), BMI ≥ 25 ($p < 0.001$), family history ($p < 0.001$) had a significant impact on T2DM patients and also being male ($p < 0.001$) older than 65 years of age ($p < 0.05$) had a significant effect on DN development. Conclusions: The results of this study suggests that T241M polymorphic variant of *XRCC3* gene is not associated with an increased susceptibility to T2DM and DN in the studied population.

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E-P07 Immunology and hematopoietic system

E-P07.01 The coinheritance of α -globin gene triplication

could explain the more profound anemia in young β -thalassemia carriers

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The clinical severity of β -thalassemia is related to the degree of α /non- α -chain imbalance. Co-inheritance of triplicated α -genes can alter the clinical and hematological phenotypes of β -thalassemia carriers. α -globin gene triplication $\alpha\alpha\alpha^{\text{anti-3.7}}$ is relatively common (~1% in the general population), but it is not systematically analyzed in beta thalassemia carriers. We have compared the average hematological parameters (Hb, RBC and HCT levels) of β -thal carriers with coinherited α -gene triplication with those of simple β -thalassemia carriers. Beta thalassemia patients with coexisting iron deficiency anemia were excluded from the study. The study population included 23 β -thal carriers with $\alpha\alpha\alpha^{\text{anti-3.7}}$ and 293 simple β -thal carriers divided into 4 groups according to type of mutation (β^+ or β^0) and their age (Table). Hb, RBC and HCT values were significantly lower in β -thal carriers with $\alpha\alpha\alpha^{\text{anti-3.7}}$ compared to simple β -thal carriers in groups 1 and 2, i.e. patients younger than 12 years, both with β^+ and β^0 mutations. No statistical significant difference was observed among β -thal carriers with and without $\alpha\alpha\alpha^{\text{anti-3.7}}$ in groups 3 and 4, i.e. older patients. Our findings suggest that the coinheritance of α -

Group (Age/ β -thal mutation)	Hematological parameter	β -thal carriers with $\alpha\alpha\alpha^{\text{anti-3.7}}$ (n = 23)	Simple β - thal carriers (n = 293)	P- value
1 (2–12/ β^+)	Hb	9,6 ± 0,4	11,3 ± 1,1	0,0012
	RBC	5,3 ± 0,5	5,7 ± 0,5	0,0420
	HCT	30,6 ± 1,9	35,8 ± 4,3	0,0102
2 (2–12/ β^0)	Hb	8,9 ± 0,7	10,5 ± 0,8	0,0001
	RBC	5,0 ± 0,3	5,7 ± 0,5	0,0001
	HCT	28,0 ± 2,1	34,0 ± 3,5	0,0001
3 (13–40/ β^+)	Hb	12,7 ± 0,9	12,7 ± 1,4	0,9943
	RBC	6,2 ± 0,4	6,1 ± 0,5	0,4919
	HCT	39,1 ± 3,0	38,1 ± 6,8	0,983
4 (13–40/ β^0)	Hb	12,1 ± 1,0	12,3 ± 1,2	0,7170
	RBC	6,1 ± 0,6	6,3 ± 0,6	0,2782
	HCT	34,7 ± 3,2	38,4 ± 7,0	0,2076

globin gene triplication could explain the more profound anemia in young β-thal carriers.

Hb-hemoglobin (g/dL); RBC-red blood cells ($10^6/\mu\text{l}$); HCT-hematocrit (%)

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E-P07.02

Violence impacts allostatic load IgE and Epstein Barr Virus levels in urban youth

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Allostatic load is the cumulative wear and tear experienced by the immune system in response to chronic environmental stressors. Many studies have observed increased allostatic load in African American populations in comparison to their European American counterparts. A key environmental stressor in the lives of young African Americans is the occurrence of violence. This violence has impacts not only in terms of criminal justice interventions, but also on the physical and mental health of African American communities. Understanding how the experience of violence contributes to allostatic load is a critical step in parsing and ultimately reducing its effect on at risk young adults. Previous work has shown that there are gender effects to the experience of violence and to cortisol concentration variation. Urban study participants ($N = 557$, women = 274, men = 283) were queried about their experience of community, interpersonal and intimate partner violence as well as assessed on key indicators of mental health state. We include five stress biomarkers typed in study participants: C reactive protein, IgG, IgE, IgA, IgM and Epstein Barr Virus Viral Capsid Antigen (EBVVCA). We find that familial violence is most correlated to elevated IgE levels ($R = 0.37$) and EBVVCA is strongly correlated to perceived anxiety with their local environment ($R = 0.87$). Naïve Bayes implemented machine learning reveals that ‘ability to cope’ responses predict elevated stress biomarker levels and allostatic load index values. These findings suggest that the internalization of violence may be more important than the actual experience of violence in predicting elevated stress biomarker levels.

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E-P07.03

(--) NOR and (aa) Aurora Borealis - two novel deletions causing alpha-thalassemia found in Norwegian patients

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Introduction: Alpha-thalassemia is one of the most common monogenic diseases worldwide and is caused by reduced or absent synthesis of alpha-globin chains, most commonly due to deletions of one or more of the alpha-globin genes. Sequence variants that alter gene expression or deletions involving upstream regulatory elements are less frequent. Alpha-thalassemia occurs with high frequency in tropical and subtropical regions of the world. For people of Northern European origin, inherited hemoglobin disorders, such as alpha-thalassemia, are extremely rare. Here, we describe two novel deletions causing alpha-thalassemia found in patients of Norwegian origin. Materials and Methods: The study patients were diagnosed during routine hemoglobinopathy evaluation carried out at the Department of Medical Biochemistry, Oslo University Hospital, Norway. The patients were selected for their thalassemic phenotype, despite Norway as country of origin. All samples went through standard hemoglobinopathy evaluation. Quantitative real-time PCR copy number variation (CNV) analysis was applied to detect uncommon deletions in the alpha-globin gene cluster. Deletion breakpoints were characterized using gap-PCR and DNA sequencing. Results: Two novel deletions, (--)NOR and (αα)Aurora Borealis, were identified in altogether nine patients from two and one families, respectively, all of Norwegian origin presenting with microcytosis. The (--)NOR deletion was a result of homologous recombination deleting both alpha-globin genes. The (αα)Aurora Borealis deletion caused alpha-thalassemia by affecting the upstream regulatory element, HS-40, leaving the alpha-globin genes intact. Conclusions: Even though inherited hemoglobin disorders are extremely rare in indigenous Northern Europeans, the possibility of a carrier state should not be ignored.

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E-P07.04**Transcriptome analysis in human anaphylaxis**

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Anaphylaxis is a life-threatening allergic reaction, mainly involves the activation of mast cells and/or basophils, followed by the release of mediators of anaphylaxis, yet the exact molecular mechanism remains poorly understood. To better characterize the mechanisms leading to potentially lethal events, analysis of global transcriptional changes in peripheral blood samples during anaphylactic reaction was performed. RNAseq based whole transcriptome characterization of total RNA from whole blood samples of 15 patients with anaphylactic reaction was performed in three different time points: at the presentation with anaphylaxis at the emergency department, 7 days and 1 month after the anaphylactic episode. Extensive characterization of differential gene expression, cell-specific transcriptional alterations, analysis of alternative splicing patterns, and functional characterization of detected alterations was performed. Whole transcriptome expression analysis revealed striking alterations of gene expression during acute anaphylaxis in comparison to 7 days and 1 month after the episode. These alterations revealed cellular movement, cell-to-cell signaling, interaction and immune cell trafficking as well as inflammatory response, as the most important mechanisms taking place during anaphylaxis. Additional comparative analysis with expression signatures of immune cells showed significant under-expression of basophil and over-expression of eosinophil signatures during anaphylactic reaction. This finding improve our understanding of biological mechanisms underlying anaphylaxis, since our data suggests the involvement of distinct immune cells, and complex signaling changes, which reflect cellular movement and interaction during anaphylaxis.

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E-P07.05**Study of the polymorphisms in genes *IL-17*, *IL-23*, *TGF β* , *ROR γ T* and *FOXP3* in Serbian patients with antiphospholipid syndrome**

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INTRODUCTION: Antiphospholipid syndrome (APS) is an autoimmune disease characterized by arterial and venous thrombosis, thrombocytopenia, fetal loss and the presence of antiphospholipid antibodies in the serum. Primary APS (PAPS) is isolated, while secondary form (SAPS) is part of other autoimmune disorders. APS has multifactor etiology with complex interplay between genetic, immune and environmental factors. We focused our investigation to the members of interleukin 23 (IL-23) - interleukin 17 (IL-17) axis, with the aim to analyze selected polymorphisms in genes *IL-17*, *IL-23*, *TGF- β* , *ROR γ T* and *FOXP3*, and to investigate correlation of genotype with corresponding cytokines' level and with clinical phenotype in APS. **MATERIAL AND METHODS:** We have analyzed 50 patients with PAPS, 50 patients with SAPS, and group of healthy controls from Serbia. The SNPs rs2275913 (*IL-17A*), rs763780 (*IL-17F*), rs11209026 (*IL-23*), rs9826 (*ROR γ T*) and rs3761548 (*FOXP3*) were genotyped by Taq-Man allelic discrimination assays, while rs1800471 (*TGF- β*) was analyzed by allele-specific PCR. Serum concentrations of IL-17, IL-23 and TGF- β were measured by ELISA method. **RESULTS AND CONCLUSION:** No statistically significant differences were observed in the distribution of genotypes and alleles of the analyzed polymorphisms in patients with PAPS and SAPS compared to the healthy subjects. The levels of IL-17, IL-23 and TGF- β were significantly higher in PAPS and SAPS patients than in the control group, but without correlations to the genotype. Considering clinical phenotype, majority of analyzed polymorphisms showed correlation with vascular manifestations of both PAPS and SAPS, while fetal loss in SAPS was associated with the T allele of *ROR γ T* polymorphism.

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E-P07.06**Whole exome sequencing identifies a novel ATM mutation resulting ataxia-teleangiectasia**

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Mutations in the ATM gene cause rare autosomal recessive diseases ataxia-telangiectasia (Louis-Bar syndrome). These diseases share cerebellar ataxia, telangiectases, severe combined immunodeficiency (affecting mainly the humoral immune response), and a predisposition to malignancy. The earlier ataxia can be misdiagnosed as ataxic cerebral palsy before the appearance of oculocutaneous telangiectases. The ATM protein is a member of the phosphatidylinositol 3-kinase family of proteins that respond to DNA damage by phosphorylating key substrates involved in DNA repair and/or cell cycle control. Patient - 4-years boy with early-onset ataxia (since 1 years), oculous telangiectases, raised alpha-fetoprotein in the blood, dysgammaglobulinemia, decreased cellular immune responses, and peripheral lymphopenia. We performed targeted sequencing of peripheral blood's DNA using Illumina HiSeq2500, NEBNext preparation protocol, Agilent Focused Exome panel and our own analytical pipeline. Variant calling and pathogenicity scoring were done based on ACMG guidelines. We identified a compound heterozygous mutation in the ATM gene on chromosome 11q22.3. The first variant is stop gained c.5932 G > T that was previously identified with frequency 0,25% in Russian population. The second variant is stop gained c.494 T > G. This variant is absent from dbNSFP, Clinvar, OMIM and HGMD pathogenicity databases, and from 1000Genomes project, ExAC and Genotek frequency databases. Capillary sequencing confirmed mutations found by NGS in proband. Also both parents were confirmed to be heterozygous carriers.

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E-P07.08

Targeted NGS for diagnosis of systemic autoinflammatory disorders

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Introduction: Systemic auto-inflammatory disorders (SAIDs) represent a heterogeneous group of diseases. Monogenic germ-line defects contribute to substantial share of SAID morbidity, however definite genetic diagnosis of SAIDs remains complicated due to scarcity of disease-specific phenotypes and high number of genes to be analyzed. Materials and methods: 18 Russian patients with non-classical clinical course of autoinflammatory disease, who were negative for mutations in the most common SAID genes (whole coding sequences of MVK, NLRP3, TNFRSF1A and exon10 of MEFV), were subjected to multigene analysis. NGS panel was composed of 302 immune-related genes, including 23 genes known to be associated with SAIDs. Results: Highly penetrant mutations in SAID genes were identified in 3 patients. In addition, 10 patients carried rare variants of unknown significance or low-penetrant pathogenic mutations in MEFV, NLRP12, NOD2, LPIN2 and other genes. Patients carrying low-penetrant mutations had milder disease course and usually did not require biologics, while those with highly penetrant mutations had more severe course and needed anti-IL-1 treatment. Conclusions: Targeted NGS is a valuable approach to genetic testing in SAIDs. Presence of low-penetrant mutations and their combinations may contribute to some cases of non-classical (undifferentiated) SAID. This work has been supported by the Russian Scientific Fund (grant number 15-15-00079).

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E-P07.10

Screening for *CALR* gene mutations in Jak-2 V617F mutation negative patients with myeloproliferative neoplasms

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Introduction: Mutations in exon 9 of *CALR* gene (OMIM *109091), encoding for the endoplasmic reticulum-associated, calcium binding protein calreticulin, have been found in patients with Myeloproliferative Neoplasms (MPNs) and considered in MPN diagnostics recently. We aimed to investigate the mutations in exon 9 of *CALR* (NG_029662.1, NM_004343) in the patients with MPNs [Essential thrombocythosis(ET), PolycythemiaVera(PV),

myelofibrosis, lymphoma and unspecified MPNs] who were negative for Jak-2 (V617F) mutation.

Materials and Methods: DNA samples from peripheral blood of 147 patients with Jak-2 negative MPNs were included. 62 of the patients were screened for *CALR* exon 9 mutations by Sanger sequencing between 03.08.2015–23.12.2016. 85 samples were retrospectively selected from patients with MPNs who were found to be negative for Jak-2 V617F mutation in our center between 02.06.2012–03.07.2014. Retrospective samples (n=85) were sequenced on MiSeq (Illumina) after Nextera XT(Illumina) protocol. In-house designed primer sets were used for polymerase chain reaction before Sanger Sequencing reactions and Nextera protocol. All the mutations found by Next Generation Sequencing were confirmed by Sanger sequencing.

Results: 17 patients defined to have *CALR* frameshift mutations (11.5%). A novel complex variation (NM_004343:c.1122_1123delinsTTGT) has been defined in one of the patients. *CALR* mutation frequency was 37.5% in ET, 13.3% in lymphoma, 3.8% in PV and 6.66% in unspecified MPN patients. *CALR* mutations were not defined in myelofibrosis subgroup.

Conclusions: *CALR* mutation frequency was highest in the ET but lowest in the PV subgroup of MPN patients as in general reports. Sequencing is helpful for defining uncommon frameshift variations of *CALR*.

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E-P07.11

Transcriptome analysis of epithelial and immune fractions clarify the mechanisms involved in celiac disease

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Celiac disease (CD), an autoimmune disorder suffered by 1% of the population, affects genetically susceptible individuals when gluten is present in their diet. Gene-expression analyses have been used to analyze candidate genes in biopsies of the duodenum, the target tissue of CD, or whole transcriptome in peripheral blood cells. However, all the transcriptomic changes that happen in the different cellular fractions involved in CD remain unclear. RNA-Seq was

used to analyze the transcriptome of complete biopsies from 4 CD and 4 controls; epithelial cell enriched fraction from 10 CD and 12 controls; and immune cell enriched fraction from 7 CD and 5 controls. Then, in each kind of cell, differential expression analyses were carried out using Cufflinks, Deseq2 and edgeR. A gene was considered as differentially expressed if at least two methods detected differential expression. In complete biopsies 1057 genes were differentially expressed; 935 in epithelial fraction; and 566 in immune fraction. Enrichment analyses showed that in the epithelial fraction the interferon signalling pathways were overexpressed and in the immune fraction the chemokine receptor pathway, while genes related to pathways such as cell cycle and transmembrane transport were only detected in complete biopsies. Although the enrichment analyses showed the usual pathways related to CD, we were able to distinguish in what fraction were changed. Thus, the analysis of each fraction is useful to clarify the role of the genes and the underlying mechanisms involved in CD; and to improve the subsequent functional experiments.

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E-P07.12

Cell-free DNA from plasma and serum differ in relative content of telomeric sequences and in inimmune response activation

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Introduction: Despite its practical exploitation (e. g. in non-invasive prenatal diagnostics), the biological meaning of cell-free DNA (cfDNA) in circulation is not understood. CfDNA is able to bind to toll-like receptor 9 (TLR9) and to contribute to immune response tuning. Telomeric sequences are able to block TLR9. **Methods:** We stimulated THP1 cells by plasma and serum samples of young healthy volunteers. For stimulatory experiments, 7 pairs of plasma and serum samples were used. For each sample the content of telomeric sequences was determined using qPCR in the form of T/S (telomere/single copy gene) ratios. A half of volume of every sample was treated by DNase. Using qPCR, we detected the alterations in TNF- α mRNA levels

before and after stimulation of THP1 cells by DNase treated and non-treated samples. Results: DNase treatment of plasma and serum samples led to significant enhancement of their stimulatory activity ($p = 0.018$ and $p = 0.028$, respectively). Significant differences were observed between non-treated plasma and serum samples ($p = 0.018$). DNase non-treated serum samples stimulated the relative TNF- α expression in higher extent than non-treated plasma samples. Conclusion: The content of telomeric sequences in plasma and serum may represent one of multiple factors contributing to the fine tuning of immune response by cfDNA. Funding: grants no. PRVOUK P25/LF1/2 and SVV 260 263 (Ministry of Education, Youth and Sport of the Czech Republic) and grant RVO-VFN64165 of the Ministry of Health of the Czech Republic.

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E-P07.15

Rare copy number variations in patients with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease with a strong genetic background characterized by chronic inflammation and autoantibody production. Copy number variations (CNVs) can contribute to the variability of the risk for complex diseases. The purpose of this study was to investigate the role of rare CNVs in SLE. Genomic DNA from 23 unrelated SLE patients and 110 healthy subjects was submitted to the genome-wide human CytoScan HD array to screen for DNA gains and losses. Detection of CNVs was performed using Chromosome Analysis Suite 3.0 and Plink v.1.9 software. Unique CNVs in SLE cohort in comparison with those identified in healthy controls were selected for evaluation of the population frequency using data from the *Database of Genomic Variants* and *HapMap* project. After filtering for unique CNVs with population frequency < 1%, *in silico* evaluation of genes overlapped by the CNVs was performed to select likely pathogenic rare

variants. These CNVs were submitted to validation using droplet digital PCR as a target-specific methodology to confirm results obtained from the array. A rare heterozygous duplication of 649 kb in chromosome 12 including five genes (*LDHB*, *KCNJ8*, *ABCC9*, *CMAS*, *ST8SIA1*) and three heterozygous deletions covering genes previously involved with susceptibility to autoimmunity (*CFHR4*, *CFHR5* and *HLA-DPB2*) were identified. This is the first report describing likely pathogenic CNVs encompassing these regions in SLE. Support: FAPESP (2016/10306-8, 2013/17062-9, 2011/23794-7), CNPq (312547/2009-9, 304455/2012-1).

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Molecular genetic study of Factor v deficiency in two Iranian families

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Factor v deficiency is a rare autosomal recessive disorder with 1 per 1000000 live birth. This coagulation disorder is caused by mutations in F5 gene. The Levels of Fv antigen and its coagulant activity determine the clinical manifestation which ranges from mild to severe. The most common symptoms of the disease include bleeding from mucosal surfaces and postoperative hemorrhages. Three Iranian families with 3 patients with severe bleeding symptoms were referred to Dr.Zeinali medical genetic laboratory for confirmation of the clinical diagnosis and carrier detection for other family members. Direct sequencing of the F5 gene exons and intron-exon boundaries was performed to identify pathogenic mutation in affected individuals. Three different mutations were identified. Two of these mutations were first reported in Iran and one of them was a novel missense mutation. Heterozygosity of the identified mutations was confirmed in the parents and was not present in healthy members of the family. There is no exact report on the number of patients in Iran. It seems that due to increased rate of consanguineous marriage, and autosomal recessive inheritance of the disease, it is expected to have higher number of patients in comparison to western countries. To date, the present study is the first report of genetic study of Factor v deficiency in Iran. Patients and their families face lots of difficulties because of the symptoms of Factor v deficiency and factor replacement which uses as the

treatment. More studies are recommended to update the mutation spectrum.

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E-P07.19

RAC1 expression and role in IL-1 β production and oxidative stress generation in familial Mediterranean fever (FMF) patients

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Familial Mediterranean fever (FMF) is a recessively inherited autoinflammatory disorder. The caspase-1 dependent cytokine, IL-1 β , plays an important role in FMF pathogenesis, and RAC1 protein has been recently involved in IL-1 β secretion. This study aims to investigate RAC1 expression and role in IL-1 β and caspase-1 production and oxidative stress generation in FMF. The study included 25 FMF patients (9 of them during attack and remission), and 25 controls. *RAC1* expression levels were analyzed by real-time PCR. *Ex vivo* production of caspase-1, IL-1 β , IL-6 and markers of oxidative stress (MDA, catalase and glutathione system) were evaluated respectively in supernatants of patients' and controls' PBMC and PMN cultures, in the presence and absence of RAC1 inhibitor. *RAC1* gene was overexpressed in patients in crises compared to those in remission or controls. Caspase-1 levels were higher in LPS-induced PBMCs of patients than controls. Spontaneous and LPS-induced IL-1 β production was comparable in patients and controls, whereas LPS-induced IL-6 production was enhanced in patients, compared to controls. However, inhibition of RAC1 resulted in a decrease in caspase-1 and IL-1 β levels, but not IL-6. LPS-stimulated PMNs produced higher MDA levels in patients than controls, but these levels were decreased in the presence of RAC1 inhibitor. The reduced catalase and GSH activities in unstimulated culture supernatants of patients compared to controls were increased in the presence of RAC1 inhibitor. Our results show the implication of RAC1 in the inflammatory process of FMF by enhancing IL-1 β production, through caspase-1 activation, and generating oxidative stress, even during asymptomatic periods.

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E-P07.20

Tradition meets high technology. Chromosome breakage analysis combined with exome sequencing in the diagnosis of Fanconi anemia

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Background Fanconi anemia (FA) is a rare bone marrow failure disorder characterized by clinical and genetic heterogeneity with at least 15 genes involved which challenges the molecular diagnosis of this rare disease. The chromosome breakage analysis is still used to establish the diagnosis of Fanconi anemia followed by mutation analysis in test positive patients. We present a patient diagnosed with Fanconi anemia using chromosome breakage analysis followed by exome sequencing and identification of two novel mutation alleles in the *FANCA* gene.

Methods Peripheral blood lymphocytes from a five-year-old boy suspected of Fanconi anemia demonstrated DNA instability when cultured with mitomycin C. DNA was subjected to exome sequencing using KAPA HTP library kit, the Nimblegen MedExome Plus capture probes and sequencing on NextSeq500 (Illumina) with average read depth 138 \times . Focused data analysis on Fanconi associated genes was performed using GATK best practice pipeline and Ingenuity Variant Analysis (Qiagen). The variants were confirmed using Sanger sequencing and MLPA.

Results Exome sequencing revealed a novel heterozygous splice variant in the *FANCA* gene (c.523-2A>G) suspected to lead to mis-splicing of exon 6 and CNV analysis on exome data revealed a deletion in the *FANCA* gene confirmed by MLPA analysis as a novel heterozygous deletion of exon 1 to 5 in the *FANCA* gene (c.(?_-227126)_ (522 + 1_523-1)del). Parental analysis confirmed that the variants are situated in trans position.

Conclusion The combination of traditional cytogenetic techniques and exome sequencing with focused data analysis on Fanconi associated genes are valuable tools in the genetic diagnosis of Fanconi anemia.

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E-P07.23

Investigation of the effect of colchicine on endoplasmic reticulum stress in FMF patients

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Introduction: Familial Mediterranean fever is a recessively inherited systemic autoinflammatory disorder originated by mutations in the MEFV gene. The protein product of MEFV named pyrin (or marenostatin). Pyrin is a negative regulatory of a specific inflammatory pathway, and that loss of Pyrin function is responsible for the enhanced inflammation seen in the FMF patients. Endoplasmic reticulum (ER) stress is a very conserved pathway that provides the cell for regulating endoplasmic reticulum (ER) stress that is caused by the secretory requirements associated with environmental forces. In this role, the ER stress has increasingly been revealed to have important roles in immunity and inflammation. Thirty patients were included in the study. Fifteen patients were examined prior to treatment and 15 during treatment with colchicine (1–2 mg/day). Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) by Trizol Reagent and standard chloroform extraction method. cDNA was synthesized from 1 µg of total RNA using Transcripter High Fidelity cDNA synthesis kit. Expression levels of genes (GRP78, CHOP, EDEM1) associated with endoplasmic reticulum stress were analyzed by RT-qPCR. Fold changes were calculated using the -ΔΔCT method. The statistical significances were estimated applying two-tailed student's t-test and analysis of variance (ANOVA). Results: When we compared the results of the analysis of the group using colchicine with the group that did not use it, we found that ER stress-related gene expression levels were a difference in the group not using colchicine. This demonstrates that colchicine is effective on ER stress in FMF patients.

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E-P07.25

Novel mutation (T742S homozygotes) in a case of Familial Mediterranean fever in an Iranian patient

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Introduction: Familial Mediterranean fever (FMF) is a recessively transmitted auto-inflammatory disease, caused by point mutations in the *mefv* gene product pyrin. Progressive systemic amyloidosis is the most important complication of FMF which usually leads to death from renal failure within a year. Subject: we carried out direct sequencing of all exons of the *mefv* gene in an Iranian Azari patient with clinical features of FMF. Family history indicated that both her brothers suffered from renal failure eventually leading to death. DNA sequencing revealed the presence of a novel mutation (T742S homozygotes) localized in exon 10. Identifying novel missense mutations domain and their functional consequences is critical to explain their role in FMF pathogenesis. Initial studies have suggested that the presence of the (M694V homozygotes) mutation carries a significant risk for the development of renal amyloidosis We explored M694V and T742S the Pyrin protein domain-level landscape which elucidate both mutations are in a B-strand structure in the c-terminal-B30.2 domains. The C-terminal B30.2 domain of pyrin is necessary and sufficient for the interaction, and binding was reduced by FMF-associated B30.2 mutations. Modeling of the crystal structure showed the pyrin B30.2 domain corroborated both the interaction and the importance of M694V pyrin mutations. Conclusion: The correlation between the M694V and T742S protein domains suggest that they will have a direct impact on ligand binding, so the new mutation can also be associated with the occurrence of renal failure.

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E-P07.26

G6PC3 Deficiency: Mind the Gap between Mild and Severe Neutropenia

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

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E-P07.27

A genome-wide association study identifies a novel susceptibility locus for the immunogenicity of polyethylene glycol

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Conjugation of polyethylene glycol (PEG) to therapeutic molecules can improve bioavailability and therapeutic efficacy. However, some healthy individuals have pre-existing anti-PEG antibodies and certain patients develop anti-PEG

antibody during treatment with PEGylated medicines, suggesting that genetics might play a role in PEG immunogenicity. We performed genome-wide association studies for anti-PEG IgM and IgG responses in Han Chinese with 177 and 140 individuals, defined as positive for anti-PEG IgM and IgG responses, respectively, and with 492 subjects without either anti-PEG IgM or IgG as controls. We validated the association results in the replication cohort, consisting of 84 and 103 subjects with anti-PEG IgM and anti-PEG IgG, respectively, and 277 controls. We identify the immunoglobulin heavy chain (*IGH*) locus to be associated with anti-PEG IgM response at genome-wide significance ($P < 1.67 \times 10^{-15}$). Our findings may provide novel genetic markers for predicting the immunogenicity of PEG and efficacy of PEGylated therapeutics.

J. Wu: E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Sanofi.

E-P07.28

Next Generation Sequencing analysis of familial Haemophagocytic Lymphohistiocytosis (HLH) related genes in Macrophage Activation Syndrome (MAS) and secondary HLH

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Introduction: Macrophage activation syndrome (MAS) is a severe complication of rheumatic disease, currently classified among the secondary forms of HLH (secHLH). Primary HLH (pHLH) is caused by mutation of genes encoding for proteins involved in cytotoxic functions. Mice carrying heterozygous mutations in more than 1 pHLH gene carry a higher risk to develop HLH following viral infection, suggesting that accumulation of partial genetic defects may be relevant in HLH. We aim at analysing, with next generation sequencing (NGS), genes involved in pHLH in MAS and in secHLH. Materials and Methods: We performed Targeted resequencing on patients using a panel including the principal HLH-related genes (*PRF1*, *UNC13d*, *STX11*, *STXBP2*, *Rab27a*, *XIAP*, *SH2D1A*) on the Illumina MiSeq® and NextSeq550® platforms. We applied *in silico* studies, using SIFT and PolyPhen softwares, only to variants with an allelic frequency $\leq 1\%$. Results: We studied 39 patients: 24 MAS and 15 with HLH secondary to infections. In 19 patients we identified at least 1 heterozygous variant in one of the analysed genes with a detection rate of 49%. *PRF1* gene was the most involved in MAS patients and *RAB27A* variants were more frequent in secHLH. All secHLH patients carrying variants in 2

different genes showed higher frequency of recurrences and disease severity compared to patients carrying one or no variants. Conclusions: Re-occurrence and disease severity of disease tend to be more frequent and more severe in patients who carry mutations in two genes. These data are consistent with the polygenic model of secHLH.

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E-P07.31

An effective molecular genetic diagnostics protocol of Hemophilia A in patients from Russian Federation

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Introduction: Hemophilia A (HA) is a frequent X-linked recessive blood clotting disorder. It affects 1 of 5000 males. HA is caused by mutations in *F8* gene located on the Xq28 chromosome. More than 3000 mutations were described in this gene. The most common mutations in patients with severe HA (FVIII:C < 1%) are: the intron 22 inversion (Inv22) - 40–45%, the intron 1 inversion (Inv1) - 2–5%, gross deletions - 2–5%. Materials and Methods: In this study we looked for Inv22, Inv1 and gross deletions in 93 families from Russian Federation with varying HA severity using IS-PCR method (Rossetti L.C., 2008) and multiplex PCR. Results: Inv22 was found in 36,6% cases. Mothers of probands with detected Inv22 (DNA were available in 8 cases) were determined to be carriers of this mutation. Standard Inv1 wasn't revealed in this cohort. A complex rearrangement resulting in intron 1 inversion and exon 2–13 duplication was found by means of additional quantitative MLPA in 1 case (1%). Gross deletions were detected through multiplex PCR in 2 cases (2,2%). Moreover, mutations of other types (small insertions, deletions and single base substitutions) were revealed by multiplex PCR in 12 cases (12,9%). The two systems' effectiveness reached 52,7%. Conclusions: The results of this study are consistent with literature data. The Inv22 frequency in Russian Federation, as well as all over the world, is very high. Using two simple and cheap detection systems on the first step of HA molecular diagnostics allows to detect it in more than half of the cases.

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E-P07.32

Investigation of *MEFV* gene mutations in hidradenitis suppurativa

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Introduction: Hidradenitis suppurativa (HS) is a recurrent, chronic disease presenting with subcutaneous nodules and abscesses mostly in axillary and inguinal areas. HS has recently been associated with autoinflammatory diseases such as familial Mediterranean fever (FMF). Mutations in the *MEFV* gene cause FMF. The *MEFV* gene codes a protein called pyrin in the autoinflammatory pathway. Because of possible association of these two autoinflammatory diseases and recent reports, we aimed to evaluate *MEFV* gene mutations among HS patients.

Materials and Methods: Consecutive 22 patients with active HS who applied to dermatology clinic in the last six months were included in the study. Patients were evaluated for Hurley stages, accompanying symptoms and autoinflammatory diseases and pedigree analysis. All exons and exon-intron boundaries of the *MEFV* gene was sequenced on the patients' DNAs.

Results: *MEFV* mutations were found on 18 of 44 alleles (40.9%). 50% of individuals had parental consanguinity. Three patients were already diagnosed with FMF. Five (22.7%) individuals were compound heterozygous. One patient was homozygous for both M694V and R202Q mutations. Six cases were heterozygous. One patient with accompanying ankylosing spondyloarthropathy and acne conglobata and another patient with recurrent pyoderma gangrenosum had compound heterozygous mutations without classical FMF symptoms.

Conclusions: This preliminary study remarks that *MEFV* mutations are seen in patients with HS-autoinflammatory spectrum diseases. Dysregulation of *MEFV*-related pathways may be responsible in some patients with HS. Homozygous and compound heterozygous patients should also be investigated and followed up for FMF manifestations.

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E-P07.33

A novel mutation of *ILR2RA* gene in

immunodeficiency 41 with lymphoproliferation and autoimmunity disorder

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Clinical Presentations: The patient was a 8 years old boy with diarrhea, vomiting and weight loss suspicious metabolic disease. He was intellectually normal and had slight delay in physical development. He is reported to have hyperthyroidism, rheumatoid arteritis, proptosis and inflammation in the retina. He is a product of consanguineous marriage and family reported another boy with similar phenotype who died early and one spontaneous abortion. **Methods:** Whole exome sequencing were used to enrich all exons of protein coding genes as well as some important other genomic regions. Next generation sequencing was performed to sequence close to 100 million reads on Illumina sequencer. Bioinformatics analysis of the sequencing results was performed using international databases and standard bioinformatics software. The observed mutation was confirmed with Sanger sequencing in proband and parents. **Results:** One deleterious novel homozygous nonsense mutation in IL2RA gene (NM_000417:exon1:c. G25T:p.G9X) was found. There was also identified another possible deleterious hemizygous missense mutation in POLA1 gene that might contribute to the observed phenotype. There was no report of these mutations in the literature. **Conclusion:** Mutation in the IL2RA gene is shown to cause immunodeficiency 41 with lymphoproliferation and autoimmunity disorder. Immunodeficiency-41 is an autosomal recessive complex disorder of immune dysregulation.

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E-P07.34

Single-tube multiplex PCR assay for the detection of the most common mutations in myeloproliferative neoplasms

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Introduction: The most common mutations associated with the major myeloproliferative neoplasms (MPNs) are JAK2 V617F, mutations in CALR and MPL genes. The detection of these mutations provides valuable information for diagnosis, prognosis and follow-up of MPN patients. Currently, the methodology for the detection of these mutations encompasses a combination of 3 different assays. The aim of this study was to develop a single tube PCR method for the detection of the most common MPN mutations. **Materials and methods:** For the development of the assay we used control DNA specimens containing known amounts of the mutant allele (0%- 75% for JAK2V617F or each of the 4 MPL mutations) prepared for QA and inter-laboratory validation within the EU-MPN network and DNA from patients with known CALR indel mutations detected by Sanger DNA sequencing. The method is based on a single tube multiplex allele - specific PCR reaction (for JAK2 V617F and MPL mutations) and size-difference (for CALR mutations) using 8 different pairs of primers labeled with different dyes followed by capillary electrophoresis. **Results:** Using this assay we obtained 100% specificity and 100% sensitivity for the detection of each of these mutations at the detection level of 1% of the mutant allele. The assay was clinically validated using DNA samples from 317 MPN patients which were previously analyzed by standard methodology. **Conclusion:** Our multiplexed PCR assay represents a sensitive, cheap, fast and easy platform for the detection of the most common mutations present in >90% of MPN patients.

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E-P07.35

Acute lymphoblastic leukemia and Klinefelter syndrome in a 5-year-old boy: case report

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Introduction: Klinefelter syndrome (KS) is a disorder affecting males characterized by gynecomastia, small testes and infertility. KS results from a constitutional extra X chromosome. It is well established that males with KS have an increased risk of malignancy, including breast cancer and germ cell tumours. However, published literature regarding the risk of haematological malignancies in males with KS are conflicting; some reports suggest an increased risk of leukaemia development, whereas others have determined it only a chance association. **Results:** We present a case of 5-year-old male patient with *de novo* T-cell acute lymphoblastic leukaemia referred to Department of Paediatric and Haematology in October 2016. The boy was initially classified into low-risk group. Since the MRD from day 15 was high (0.54%), the patient was reclassified to the intermediate risk group. The hematological remission was achieved after completion of the induction protocol. Karyotype and FISH analysis performed from the bone marrow sample determined patient's karyotype as 47,XXY,del(5)(q31),add(7)(p?), +8,del(9)(p21),der(12)?,add(16)(q?), mar1-3,-2[10]/46,XXY[7].nuc ish (D5S23,D5S721x2), (EGR1x1)[120/200].nuc ish (CDKN2Ax1,CEPx2)[80]/(CDKN2Ax0,CEPx2)[80].nuc ish(TCRx2)[200]. The additional copy of X chromosome in all analyzed metaphases raised a possibility of KS, thus to assess whether or not this is a *de novo* change, karyotyping and FISH was performed on blood sample and the result was: 47,XXY[37].nuc ish (CEPx2,CEPYx1)[200]. **Conclusions:** Scarce literature reports exist regarding the association between KS and ALL in children. The complexity of our patient's karyotype, involving chromosomal rearrangements, deletions of *EGR1* and *CDKN2A* genes, but no rearrangement in *TCR* gene is challenging in treatment choice and prognosis and requires further follow-up.

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E-P07.36

LACC1 mutations in familial form of juvenile idiopathic arthritis

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Objective: Juvenile idiopathic arthritis (JIA) is the most common rheumatologic disease of childhood. It is mostly known as a complex disorder caused by the convergence of genetic and environmental factors. We aimed to identify the putative gene responsible for the disease in familial forms.

Methods: Seven Turkish families with two to four affected members (17 in total) were chosen for the genetic analyses. We performed linkage analysis followed by candidate gene approach in two of the families, and in five families, the candidate gene was analysed for mutations.

Results: The disease gene was mapped to 13q13 with a cumulative maximal LOD score of 4.81. The region harboured *Laccase (multicopper oxidoreductase) domain-containing 1 (LACC1)* gene, relevant to the disease. Mutational analysis of all coding exons revealed homozygous severe mutations in three families: c.3 G>A (p.Met1?), c.1240 C>T (p.Arg414Ter) and c.988_990delATT (p.Ile330del).

Conclusion: In the three families with *LACC1* mutations, JIA is a monogenic disease with inter- and intra-familial clinical variability. Protein studies can reveal whether *LACC1* mutation underlies the disease in the remaining four families. The families exhibited the clinical heterogeneity typical for the disease. Our results establish that *LACC1* mutations can cause recessive systemic, oligoarticular and polyarticular JIA as recently reported in three families. This study was supported by TÜBİTAK (Grant No 114Z829).

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E-P07.37

Whole exome sequencing of laser-dissected plasma cells for identification of genetic alteration of light chain amyloidosis

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Introduction: Light chain (AL) amyloidosis is a plasma cell disorder characterized by a clonal population of plasma cells that produce monoclonal immunoglobulin chains that are deposited in tissues. In AL amyloidosis patients,

immunoglobulin is deposited in tissues before a large tumor burden develops; therefore, AL amyloidosis patients typically do not present with overt multiple myeloma (MM) at the time of diagnosis. In this study, we performed whole exome sequencing (WES) of bone marrow (BM) and peripheral blood (PB) cells and laser-dissected plasma cells. Materials and Methods: 10 AL amyloidosis, and 2 MM patients were included.. After extraction of DNA from total nucleated cells of BM and PB samples, WES was performed using Illumina HiSeq 2000. In addition, laser microdissection (LCM) was performed for 5 patients for sorting of >200 plasma cells. Results: No previously reported hotspot mutations in hematologic malignancies were found in 10 AL amyloidosis patients by WES of BM cells. We intensely investigated for the presence of 95 variants of AL amyloidosis that was recently reported, however, none of these mutations were found. In our patients, non-recurrent variants were found in each case in genes including *GBP4*, *SH2D4A*, and *AR*. In addition, plasma cells obtained from LCM, non-recurrent mutations found in each case including *HIST1H4I*. Conclusions: Our results was concordant with previous studies of WES of AL amyloidosis in that unifying mutation by WES is absent in AL amyloidosis. To elucidate pathogenesis of AL amyloidosis, deeper and broader genetic investigations beyond exome may be needed.

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E-P07.38

Mevalonate kinase deficiency in the Czech Republic

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Introduction: Disease phenotype of mevalonate kinase deficiency (MKD) varies in relation to the extent of enzymatic activity reduction from multi-organ involvement with fatal outcome in mevalonic aciduria to variably severe periodic fever of hyper-IgD syndrome. Materials and Methods: 13 patients detected between Jan 2004 and Dec 2016 were retrieved and their electronic records reviewed by treating physicians. Urinary mevalonate was assayed by gas chromatography-mass spectrometry. Analysis of the MVK gene (12q24, NM_000431.3) was performed using Sanger sequencing method. Results: The frequencies of gastrointestinal and joint symptoms were 54% and 62%, respectively. Severe symptoms (high episode frequency, splenomegaly and renal amyloidosis) that lead to the introduction of biologics were presented in 7 patients. Urine from fever episode was positive for mevalonic acid (range 2 - 127 mg/g creatinine, N < 0.1) in 11 patients, while the urine from afebrile interval was always negative. Mutations were identified in both alleles in all 13 patients. Three large deletions were found. The most common point mutations (87.5% alleles) were p. V377I (11/21) and p.R40L (6/21). Conclusions: With molecular genetic method all Czech patients were diagnosed. Assessment of urine mevalonate may serve an important screening test in patients suspected of MKD mainly at places where genetic testing availability is limited; however urine from fever episode is needed. Supported by MH CZ - DRO VFN64165 and OPPK CZ.2.16/3.1.00/24012.

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Refractory Anemia with Ring Sideroblasts (RARS): assessment of mtDNA mutations

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Background Myelodysplastic syndromes (MDS) are a heterogeneous group of bone marrow disorders characterized by ineffective hematopoiesis with cytopenias and risk of developing acute myeloid leukemia (AML). RARS is a subtype of MDS characterized by isolated erythroid dysplasia with 15% or more of the erythroblasts in the bone marrow containing iron-laden mitochondria forming a ring around the nucleus.

Aims and methods The aim of the present study is to assess the presence and significance of any alterations of the mitochondrial genome in the pathogenesis of RARS. We evaluated a total of five patients with RARS diagnosis. After clinical diagnosis, Perl’s staining, karyotyping and MLPA assay were performed on bone marrow. Total DNA was extracted from CD34, bone marrow, peripheral blood and buccal brushing cells and mitochondrial DNA (mtDNA) was Sanger sequenced.

Results Several mtDNA mutations were found in all patients but particularly in one, a 56 years old man, who early progressed in RAEB2/AML. We found homo- and heteroplasmic mutations at different proportions in the analyzed cell types.

Conclusion / Summary A possible role of the mtDNA mutations including the novel ones which might be specific of CD34 cell population will be discussed as possible contributing factor to the disease to establish whether mtDNA might represent a novel molecular marker for the diagnosis and/or prognosis of RARS.

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Potential risk of myeloid neoplasms in people living in areas contaminated with depleted uranium

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Introduction: Depleted uranium (DU) is widely used in ammunition in modern warfare. During the Bosnian war (1992–1995), DU ammunition was used in Hadzici area, where DU was measured in soil, air and water. Materials and methods: We collected clinical and genetic data on hematological malignancies in the Hadzici (DU-contaminated) and Ilijas (control) areas from 1996 to 2015. Results: We found 717 patients with hematological disease (437 from Hadzici; 280 from Ilijas). No increase in Hodgkin lymphoma, non-Hodgkin lymphoma, and multiple myeloma was noted. There were 66 patients with myeloid malignancies (44 in Hadzici and 22 in Ilijas). We assessed clinical and genetic risk factors in the analyzed patients. Compared to European age-adjusted incidences per 100,000/year, MDS, CML, and MPN incidences were 5.3, 3.4, and 2.4 times higher, respectively. Our data suggests that longer chronic exposure produced higher number of patients. The median age at diagnosis of MDS, CML, and AML patients from Hadzici was significantly lower compared to Ilijas ($p < 0.05$). 22% of AML patients from Hadzici achieved complete remission after first treatment, compared to 67% in Ilijas ($p < 0.05$). Overall survival at 24 months was 22% for Hadzici patients vs 50% for Ilijas ($p < 0.05$). Conclusion: We found an increased frequency and more severe evolution of myeloid neoplasms in the DU-stricken area of Hadzici, compared to the control area and Europe.

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The Role of *NOL7* Gene in All Trans Retinoic Acid Resistance of Acute Myeloid Leukemia Cells

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PML-RARA fusion gene is present in 98% of patients with acute promyelocytic leukemia (APL), the M3 subtype of acute myeloid leukemia (AML). All-trans retinoic acid (ATRA) is widely used to treat APL patients. *NOL7* is a tumor suppressor gene regulated by RAR-RXR. 6p23 region where *NOL7* gene is located, is associated with many cancers, including AML. We aimed to investigate the effect of *NOL7* gene on the APL cell lines (PML-RARA positive NB4 cell line and PML-RARA negative HL-60 cell line)

and the role of *NOL7* in resistance to ATRA. For this purpose, *NOL7* gene expression was downregulated by using *NOL7* siRNA and this cells were stimulated with ATRA. Apoptosis, cell viability, proliferation and granulocytic differentiation analyses were performed. We examined expression of CD11b, a granulocytic differentiation marker, by FACS analysis. Our findings showed that ATRA-induced granulocytic differentiation was blocked in the *NOL7* knockdown NB4 cells. Granulocytic differentiation was increased proportionally with *NOL7* gene expression at 48 and 72 h. In conclusion, *NOL7* expression contributes to ATRA-induced granulocytic differentiation. This work was supported by Scientific Research Project Coordination Unit of Istanbul University. Project number: 52616

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NOL7 Expression and CD11b Levels in NB4 Cells

	24 h CD11b (%)	24 h <i>NOL70</i> Expression (%)	48 h CD11b Expression (%)	48 h <i>NOL7</i> Expression (%)	72 h CD11b Expression (%)	72 h <i>NOL7</i> Expression
Control	1.06	8.70	2.46	4.59	0.61	4.32
Control + ATRA	30.28	10.35	72.92	5.93	75.10	6.63
NT siRNA	0.29	9.74	10.99	7.91	4.40	4.94
<i>NOL7</i> siRNA	0.85	1.79	8.10	2.35	4.04	3.11
<i>NOL7</i> siRNA + ATRA	1.34	1.58	36.29	1.80	61.89	2.22

CXCL10 in CXCL9 genetic variants in children with Periodic fever syndrome with aphthous stomatitis, pharyngitis, and adenitis (PFAPA)

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Introduction: Periodic fever syndrome with aphthae, pharyngitis and adenitis (PFAPA) represents the most common periodic fever syndrome of childhood with unknown etiology. PFAPA is considered as a sporadic disease although many studies suggest that it might have a genetic cause. Serum levels of chemokines CXCL10 and

CXCL9 are significantly increased in PFAPA patients during febrile state compared to PFAPA patients in afebrile state, patients with other hereditary periodic fever syndrome during flare and healthy controls.

Objective: To determine whether genetic variants in *CXCL10* gene and *CXCL9* gene are involved in PFAPA pathogenesis.

Patients and methods: We performed genetic testing on children diagnosed with PFAPA, who were followed at the University Children's Hospital, Ljubljana for 6 years. All 4 coding exons, 5'UTR, 3'UTR and promoter region of both genes were directly sequenced.

Results: Genetic analysis was performed in 62 patients. 39 patients were male and 23 were female. Mean age at the syndrome onset was 2.1 years and at diagnosis 4.2 years. Variants found are listed in table 1. Table 1

Conclusion: Variant c.-201G > A in *CXCL10* gene is listed in Human Gene Mutation Database as a disease-associated polymorphism. Functional analyses in one study showed that this variant alters the binding affinity of nuclear protein and regulates *CXCL10* expression. Therefore it could have an impact on autoinflammatory process in PFAPA syndrome. Slovenian Research Agency Grants L3-4150 and P3-0343.

Gene	Variant	rs number	No of patients	No of patients	MAF EU / (het.)	MAF global / (hom.)	MAF patients
<i>CXCL10</i>	c.-201G > A	rs56061981	8	1	0,03 / 0,11 / 0,08		
	c.61 + 125_61 + 128delAATA	rs1466747362	0	0	0,04 / 0,01 / 0,02		
	c.61 + 33 A > G	rs4241578	34	21	0,54 / 0,61 / 0,61		
	c.279-36 C > G	rs4859584	25	30	0,48 / 0,6 / 0,69		
	c.*140 G > C	rs3921	25	30	0,49 / 0,31 / 0,69		
	c.*321delC	rs34836828	25	30	0,49 / 0,31 / 0,69		
<i>CXCL9</i>	c.-127T > C	rs2276885	23	10	0,16 / 0,23 / 0,35		
	c.*94 G > C	rs1151166043	0	0	0,02 / 0,01 / 0,02		
	c.*180 A > G	rs10031051	3	0	0,02 / 0,04 / 0,02		

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E-P07.43**Decreased Severity of Experimental Arthritis in Peptidylarginine Deiminase Type 4 Knockout Mice**

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Objective. Previously, peptidylarginine deiminase type 4 (PADI4) was identified as a susceptibility gene for Rheumatoid arthritis (RA) by genome-wide association studies. PADI4 is highly expressed in bone marrow, macrophages, neutrophils, and monocytes. Peptidyl citrulline is an interesting molecule in RA because it is a target antigen of anti-citrullinated peptide antibodies (ACpas), and only PADs (translated protein from PADI genes) can provide peptidyl citrulline via modification of protein substrates. The aim of this study was to evaluate the importance of the PADI4 gene in the progression of RA. **Methods.** We generated Padi4 knockout (Padi4^{-/-}) DBA1J mice. Padi4^{-/-} DBA1J and wild-type mice were immunized with bovine type II collagen (CII) to develop collagen-induced arthritis (CIA). Expression of various inflammatory cytokines and Padi genes in immune cells was detected by real-time TaqMan assay. Cytokine concentration in sera was measured by enzyme-linked immunosorbent assay. Localization of PAD4 and PAD2 protein was indicated by immunohistochemistry. **Results.** We demonstrated that the clinical disease score was significantly decreased in Padi4^{-/-} mice and Padi4 expression was induced by CII immunization. In Padi4^{-/-} mice sera, serum anti-type II collagen (CII) IgM, IgG, and inflammatory cytokine levels were also significantly decreased compared with those in wild-type mice sera. Interestingly, Padi2 expression was compensationally induced in CD11b+ cells of Padi4^{-/-} mice. **Conclusion.** On the basis of these studies, it appears that Padi4 enhances collagen-initiated inflammatory responses. Our results revealed that PAD4 affected on expression of various cytokines and also controlled Padi genes.

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E-P07.45**A streamlined workflow for routine gene expression profiling of single cells**

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Introduction: Recent technological advances in sequencing has enabled whole transcriptome analysis (WTA) of single cells, expanding our appreciation for the high level of heterogeneity in cell lineage and function. However, single cell WTA studies are often cost prohibiting for high throughput analysis. Moreover, a significant portion of sequencing reads consist of high expressing house-keeping genes and genes of low variability. In this study we describe a comprehensive workflow of using WTA data to design a primer panel for highly multiplexed PCR.

Material and Methods: Using BD™ Resolve's single cell platform, we captured thousands of cells from dissociated mouse spleen. Around 1000 cells were subsampled for WTA. Using data generated from WTA we designed a gene panel consisting of 482 primers for immune cell related genes, using an algorithm that selects optimal polyA sites to use for individual transcripts. We then tested the custom gene panel on ~1000 cells subsampled from the original sample by highly multiplexed PCR.

Results and Conclusions: Based on gene expression, we were able to reconstruct all the major immune cell compartments as well as rarer subsets in both WTA and Targeted assays. With significantly fewer reads in the targeted library, we achieved higher capture efficiency and detected low abundance transcripts that weren't found in the original WTA. This work flow, which leverages pilot WTA data to identify highly variable genes and design primers for subsequent transcriptional profiling of similar samples, is a comprehensive and cost effective approach to study tissues at the single cell level.

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E-P07.46**Mutational landscape of thalassemia in Armenia**

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Background. Compared to neighboring countries, there are no mild or severe forms of thalassemia in Armenia. However, being in the cross of geographic areas with high frequency of the disease, the genetic diagnosis of cases with occasional hematological findings is of high importance to check the carrier state for further management and for family planning.

Methods. Genetic screening of 22 beta globin gene and 21 alpha globin gene mutations common for the Indo-Mediterranean region was performed for 98 sporadic and familial cases suspected for thalassemia intermedia or thalassemia minor.

Results. 67 patients were diagnosed as simple heterozygotes for b^0 or b^+ globin gene mutations responsible for thalassemia minor or intermedia with specific hematological features. 10 cases were detected with alpha single or double gene deletions. Four patients with intermediate beta-thalassemia were detected with simple heterozygous beta globin gene mutations with co-inheritance of triplicated alpha globin gene rearrangement. All five families involved in the study were detected with alpha or beta globin gene mutations while only one familial case with typical thalassemia intermedia was detected with beta globin compound heterozygous genotype.

Conclusion. This study reconfirms the previous reports on distribution of asymptomatic or intermedia thalassemia in Armenia. 75% detection rate of mutations emphasizes the importance of provision of genetic testing among patients with suspected phenotype. Further larger screening would better assess the spectrum of mutations identified in heterozygous beta-thalassemia cases and factors explaining clinical-molecular relationships.

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A novel Bruton's tyrosine kinase mutation in patient with X-linked agammaglobulinemia

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Background X-Linked Agammaglobulinemia (XLA) is the major primary immunodeficiency in which the body is unable to produce the antibodies responsible for the defense against bacteria and viruses. XLA (frequently called Bruton's Agammaglobulinemia) is caused by mutations in

Bruton's Tyrosine Kinase (*BTK*) gene, leading to abnormal B cells development. Here we want to report a case of Bruton's Agammaglobulinemia that was caused by new deletion in *BTK* gene. Case presentation The patient (a 6-month-old boy) was born at term to non-consanguineous parents. No family history of PID. Both parents and older sister are clinically healthy. He was breastfed till 3 months. At 3, 5 months of age he presented acute viral infection with glue ear. At 4 month - serous meningitis, at 5 month - laryngotracheitis and serous meningitis. The levels of immunoglobulins were decreased for Ig A and IgG isotypes. The virtual lack of CD19 + B-lymphocytes was defined. Additionally there was found a complete absence of KREC (kappa-deleting recombination excision circle) in dried blood spot (retrospectively). Intravenous immunoglobulin therapy was started at 6 month of age. Results The molecular diagnostics of coding region of the *BTK* gene was performed, confirming the clinical diagnosis of XLA. DNA sequencing analysis of patient showed a 13-bp deletion in exon 2 (c.64_76delCCTCTAAACTTCA), leading to occurrence of frameshift and premature termination codon (p.Pro22fsTer28). This mutation was not described earlier. The mother and the sister of proband showed heterozygosity at the same position. Conclusions Prenatal diagnostic testing has become available to this family for next pregnancy.

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E-P08 Intellectual Disability

E-P08.01

15q11.2 BP1-BP2 microduplication in a patient with intellectual disability and minor anomalies

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Introduction: Complex neurodevelopmental disorders are a group of conditions with impairments of the development and growth of the central nervous system. It could be associated with aberrations of certain chromosomal regions. 15q11.2-q13 region includes five recurrent breakpoints (BP1 to BP5), of which the most common rearrangements are BP1-BP3 and BP2-BP3 deletions. Involvement of BP1-BP2 deletions or duplications of the 15q11.2 region - including *TUBGCP5*, *CYFIP1*, *NIPA2* and *NIPA1* genes -

in neurodevelopmental disorders is less frequent in clinical practice. Materials and Methods: We have investigated a Hungarian boy with intellectual disability and minor anomalies using Agilent Human Genome Unrestricted G3 ISCA v2 Sureprint 8 × 60 K oligo-array. Results: Array-CGH examination of the patient revealed a 0.3 Mb (22,765,628–23,080,961) copy number gain of the 15q11.2 BP1-BP2 chromosomal region. The affected chromosomal region includes only the four highly conserved genes *TUBGCP5*, *CYFIP1*, *NIPA1* and *NIPA2*. Conclusions: Our results with the limited literature data discussing 15q11.2 BP1-BP2 duplications confirmed that additional copies of *TUBGCP5*, *NIPA1*, *NIPA2* and *CYFIP1* genes could lead to the development of intellectual disability and minor anomalies. Array-CGH results of our patient contributed to the establishment of genotype-phenotype correlations regarding this rare chromosomal alteration and can greatly facilitate the clarification of the function of *TUBGCP5*, *CYFIP1*, *NIPA1* and *NIPA2* highly conserved genes in 15q11.2 BP1-BP2 region.

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A 669Kb deletion in 17q23.2, encompassing *TBX2* and *TBX4* genes, in a girl with a moderate developmental delay without any other pertinent abnormality

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Microdeletion of the 17q23.1-q23.2 region recently emerged as a syndrome (OMIM#613355) based in a small number of cases with a common phenotype including mild-to-moderate developmental delay, heart defects, microcephaly, postnatal growth retardation, and hand, foot, and limb abnormalities. All patients reported to date present mild to moderate developmental delay, in particular speech delay, and half of them hearing loss. The smallest overlapping region has approximately 2.2 Mb and includes the transcription factors *TBX2* and *TBX4* genes. These genes have been implicated in a number of developmental pathways, including those of the heart and limbs. The *TBX4* gene is also associated with the autosomal dominant small patella syndrome (SPS, OMIM 147891). Here we report a 8

year-old girl with moderate developmental delay including learning disabilities. The test for Fragile X syndrome indicated an allele within the grey area (number of repeats ~50 CGG) inherited from her mother and probably not relevant. Affymetrix Cytoscan HD chromosome microarray analysis was performed and a 669 Kb interstitial deletion was detected at 17q23.2 region, encompassing only five OMIM genes: *BCAS3*, *TBX2*, *TBX4*, *NACA2* and *BRIP1*. To our knowledge this is the smallest deletion described in this region. None of the genes present in the deleted region are known to be associated with developmental problems. We compare our patient with the other similar reported cases, in order to add some increased value to the phenotype-genotype correlation of deletions in this region.

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Developmental delay, congenital heart defect and cleft palate in a patient with 1q22q23.1 microdeletion

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Introduction: Many studies have shown that molecular karyotyping is an effective diagnostic tool in individuals with intellectual disability/developmental delay. We report on a patient with novel 1q22q23.1 microdeletion, a possible cause of developmental delay and multiple congenital anomalies. Materials and Methods: A female, 6 years of age, was a first child of healthy non-consanguineous parents (DECIPHER 338439). The proband was born at gestational age of 40 weeks by Caesarian section because of breech presentation and oligohydramnios. She had low birth weight, cleft hard and soft palate, atrial septal defect and patent ductus arteriosus. Her psychomotor development was delayed. At age of 6 years her phenotype was remarkable for microcephaly, short stature, upslanted palpebral fissures, strabismus, thin upper lip, protruding ears, and camptodactyly of 4th–5th fingers. Brain MRI showed hypoplastic corpus callosum. She had severe global developmental delay, stereotypic motor behaviors and hyperactivity. Results: Whole-genome genotyping analysis using the HumanCytoSNP-12v2.1 BeadChips (Illumina Inc., San Diego, CA, USA) revealed 1q22q23.1microdeletion, 1.6 Mb in size. Real-time PCR analysis of the proband and her parents confirmed the deletion in the proband and revealed its *de novo* origin. The deletion involves *APOA1BP*,

LAMTOR2, *LMNA*, *NTRK1*, *PRCC*, *RIT1* and *SEMA4A* morbid genes. *RIT1* and *LMNA* genes are associated with Noonan and Malouf syndromes, respectively, and partly explain the clinical features of the patient. Conclusions: This presentation provides clinical and molecular characterization of previously unreported 1q22q23.1 microdeletion. Additional patients with similar microdeletions will contribute to further delineation of this rare chromosomal alteration.

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Novel recessive mutations in the *AP4M1* gene in a patient with AP-4 deficiency syndrome

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Introduction: The recently proposed adaptor protein 4 (AP-4) deficiency syndrome comprises a group of congenital neurological disorders characterized by severe intellectual disability, neonatal hypotonia that progresses to spastic paraparesia/tetraparesia, microcephaly, and limited speech. AP-4 is a component of intracellular transportation of proteins that is thought to play an important role in neurons. Recently, mutations in genes affecting all four subunits of AP-4 (*AP4M1*, *AP4E1*, *AP4S1*, *AP4B1*) have been found to cause autosomal recessive AP-4 deficiency phenotype. Materials and Methods: Disease-associated genome sequencing was performed in a 10-year-old boy born to non-consanguineous parents of Polish origin. The child presented with severe intellectual disability, neonatal hypotonia, spastic paraparesia, microcephaly, absent speech, seizures, and early onset obesity; MRI showed delayed myelination. Results: Disease-associated genome sequencing revealed the presence of two novel heterozygous variants in the *AP4M1* gene: c.566del, p.(Leu189fs) and c.916 C > T, p.(Arg306*) in the proband. Sanger sequencing analysis of the parents showed the independent segregation of the two identified alterations. Conclusions: We describe a patient with novel *AP4M1* mutations and a phenotype compatible with AP-4 deficiency syndrome. The uncommon feature in our patient is early onset obesity described so far in only one family with AP-4 deficiency. To the best of our knowledge, our report represents the first non-consanguineous family with compound heterozygous

mutations in the *AP4M1* gene. All 8 previously described consanguineous families carried homozygous *AP4M1* mutations. The study was financed by NCN Project Harmonia 4 No. UMO-2013/08/M/NZ5/00978.

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E-P08.09

A case of syndromic intellectual disability and dysmorphic features associated with a heterozygous intragenic deletion of exon 5 in ARID1B

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The ARID1B gene is involved in neural development via a post-mitotic chromatin remodeling mechanism that occurs as neurons exit the cell cycle and become committed to their adult state. Mutations in ARID1B have been shown to cause syndromic and non-syndromic intellectual disability. Intragenic deletions have been previously described in patients with ID, speech delay hypertrichosis and dysgenesis/agenesis of the corpus callosum. We describe a patient with intragenic ARID1B deletion who has developmental difficulties dysgenesis of corpus callosum and dysmorphic features. This ten years old girl came first to our clinic at the age of three years, because of developmental delay coarse facies and macrocephaly. Developmental assessment showed low-normal intelligence. She has frontal bossing, large mouth, bushy eyebrows, thin hair, myopia, and astigmatism. Brain MRI demonstrated short and thick corpus callosum and arachnoid cyst. At ten years head circumference was 57.5 cm (+ 4.0 SD), she has slurred speech and poor social behavior. CMA was done at the age of 4 y and was normal. Exome sequencing did not reveal relevant variant. At the age of ten years she was reevaluated and CMA has been repeated. A microdeletion of 144 kb in 6q25.3 was found. This deletion encompasses exon 5 in the ARID1B gene. Isolated exon 5 deletion in ARID1B has not been reported yet in association with ARID1B phenotype. This girl has Intellectual disability, speech delay and coarse facies. She also has some minor features and dysgenesis of the corpus callosum. This case emphasizes the importance of follow-up and reanalysis.

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E-P08.10

The HHID syndrome of hypertrichosis, hyperkeratosis, abnormal corpus callosum, intellectual disability, and minor anomalies is caused by mutations in *ARID1B*

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In 2004, Pöyhönen et al. reported on three unrelated patients with hypertrichosis, hyperkeratosis, abnormal corpus callosum, intellectual disability (ID), and minor anomalies including low anterior hairline, thick arched eyebrows, broad nasal tip, columella below alae nasi, short philtrum, thick everted lower lip, simple posteriorly angulated ears, and broad feet and finger tips. This observation was recognized as an OMIM entity (OMIM 609943), hereafter referred to as HHID syndrome. Only one further individual with suspected HHID has been published and the cause of their condition remained elusive. We now used whole exome sequencing (WES) in the three patients published by Pöyhönen et al. and found deleterious de novo *ARID1B* mutations as underlying cause in 2 of 3 patients. Haploinsufficiency of *ARID1B* was recently implicated in both, nonsyndromic ID and Coffin Siris Syndrome (CSS, OMIM #135900). Retrospectively, the HHID patients' phenotypes fit well into the published *ARID1B*-associated clinical spectrum including the key features of ID, hypertrichosis, abnormal corpus callosum, and coarse face. However, our patients show only mildly diminished nail size and demonstrate that the key feature of hypertrichosis vanishes to levels of normal variation during adolescence. Moreover, the most distinctive feature shared by all patients with suspected HHID is the ectodermal sign of hyperkeratotic plaques which has not yet been reported in any patient with CSS or *ARID1B*-associated nonspecific ID. This might therefore constitute either an underreported or an infrequent

but distinct novel feature of *ARID1B*-associated phenotypes.

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TBL1XR1 de novo deletion at 3(q26.32) in a boy with developmental delay, growth retardation and dysmorphisms

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Array-Comparative Genomic Hybridization(array-CGH) has the power to study the whole genome with a higher resolution, detecting imbalances that can range from single gene or exon imbalances to chromosome segments or entire aneuploidies. In patients with a specific phenotype, the identification of a single gene imbalance, namely a deletion, allows the identification of disease-related genes and genotype-phenotype correlations. *TBL1XR1* mutations have been reported to be associated with autism, autosomal dominant mental retardation(OMIM: 616944) and Pierpont syndrome(OMIM: 602342). Only two cases with gene deletions have been reported: a single case with mild developmental delay(DD) and a 1.6 Mb *de novo* deletion at 3q26.31q26.32; and a familial case (mother/daughter) with moderate intellectual disability(ID) and facial dysmorphisms with a 708 kb deletion at 3q26.32. We report a 5½ year old male with global DD, growth retardation and dysmorphisms with a 3q26.32 *de novo* deletion, being *TBL1XR1* the only known coding gene within the deletion. *TBL1XR1* encodes a ubiquitously expressed protein that localizes to the nucleus and plays a role in transcription mediated by nuclear receptors. *TBL1XR1* mutations have also been described in autistic patients with ID. In Decipher Database, deletions encompassing *TBL1XR1* are reported in two patients: one with a *de novo* deletion and autism, hearing impairment and spotty hyperpigmentation, and the other with a partial *de novo* deletion of the gene but other genomic imbalances. The fact that the gene presents a high

haploinsufficiency score, together with the report of patients with ID and *de novo* *TBLIXR1* deletions, supports its involvement in human disease.

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ArrayCGH study of Romanian patients with intellectual disability, developmental delay and malformations

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Chromosomal aberrations are a common cause of intellectual disability (ID) and malformations. Array comparative genomic hybridization (arrayCGH) is a powerful tool for detecting relative small genomic imbalances and adds value to the cases with structural chromosomal aberrations identified by conventional cytogenetics. In our ongoing study we so far included 57 children aged between 1–11 years, referred for dysmorphic features, developmental delay, ID, different malformations. All patients had conventional karyotype performed. Also ten parents were included in the study. We used SurePrint G3 ISCA V2 CGH 8 × 60 K Array Kit (Agilent Technologies), NimbleGen MS 200 Microarray Scanner and NimbleGen MS 200 Software v1.1. Data analysis was done with Agilent CytoGenomics Software and relevant databases were consulted. CNVs detected were confirmed by MLPA (Multiplex Ligation-dependent Probe Amplification). Our arrayCGH analysis accurately characterized the aberrations in 19 cases with abnormal karyotype. In other 16 cases arrayCGH identified CNVs (copy number variations) that might explain or contribute to the phenotype of patients with normal conventional karyotype. ArrayCGH revealed no pathogenic genomic CNVs in 4 cases, suggesting that the aberrations found in the karyotype were balanced, or encompassed heterochromatic regions. The results of our study sustain the contribution of arrayCGH in postnatal diagnosis as it can delineate the genomic imbalances; help

in unraveling the genes involved in pathogenesis of congenital anomalies and provide a correlation to patient’s phenotype. Furthermore, the molecular characterization of the alterations provides valuable information for the follow-up of the patients and genetic counselling of their families. Funding: PN-II-PT-PCCA-2013-4-133 grant.

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E-P08.13

A missense mutation in the ARX gene in a family with X-linked non-syndromic mental retardation: Genotype-phenotype correlation incl. functional assays

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Introduction: The ARX (Aristaless-related homeobox) gene belongs to a large family of homeodomain transcription factors critical to development. Mutations in *ARX* cause X-linked intellectual disability. The phenotypic spectrum comprises a series of X-linked developmental disorders ranging from lissencephaly to agenesis of the corpus callosum with abnormal genitalia to infantile spasms without brain malformations to syndromic and non-syndromic mental retardation. **Material and Methods:** We describe a five-generation Danish family with three males at the age of 18, 44, and 61 years, showing mental retardation. Except from large ears, clinical evaluation did not reveal any distinct facial dysmorphic features. Whole exome sequencing with targeted analysis of the coding regions of the X chromosome was carried out in two of the affected males, a suspected obligate carrier female, and a healthy male. **Results:** The filtering process revealed a novel variant, NM_139058.2:c.1A>G, in the first codon of *ARX*. Recently, premature termination mutations very proximal in the *ARX* gene have been reported to lead to reinitiation of translation and produce a truncated protein at markedly reduced level in correlation with a less severe phenotype. **Conclusions:** The finding of a mutation in the first codon of the *ARX* gene in a family segregating non-syndromic mental

retardation supports the observed genotype-phenotype correlation reported for mutations proximal in *ARX*. We have initiated a lymphoblastoid cell line and are in the process of performing a functional assay to investigate the observed genotype-phenotype correlation. Grants: None

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E-P08.14

Maternally inherited deletions in 7q31 encompassing *CADPS2* in two unrelated patients with intellectual impairment suggesting a parent-of-origin effect

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CADPS2 plays an important role in the release of neuropeptide-Y and brain-derived neurotrophic factor (BDNF). BDNF is indispensable for brain development. *CADPS2* is maternally expressed in human blood and amygdala. Maternally inherited genetic variants of *CADPS2* were suggested to contribute to intellectual disability (ID) and autism spectrum disorders (ASD), whereas paternally inherited missense variants were not shown to have specific phenotype. We report on two unrelated girls, in whom array-CGH was performed due to ID and behavioral problems. Patient 1, a 13-year-old girl showed a microdeletion of 79 kb in 7q31.32(12212618_122291639) (GRCh37/hg19) encompassing four exons of *CADPS2*. Her behavioral phenotype was characterized by anxiety. She showed mild dysmorphic features in terms of arched eyebrows and a prominent columella, the body measurements were normal. Patient 2, a 4-year-old girl, had a microdeletion of 4.15 Mb in 7q31.31q31.32(118243718_122394183) (GRCh37/hg19) encompassing the whole *CADPS2* gene. This patient showed behavioral problems consisting of impulsive disturbance with a tendency of self-destruction. She had no distinctive dysmorphic features but shows a secondary microcephaly (-4.5 SD). Both deletions were of maternal origin. Until now only three patients with ID and ASD of

two families were reported to have deletions of *CADPS2*, one *de novo* and one maternally inherited. We report on two new unrelated patients with maternally inherited *CADPS2* deletions. Our findings support the hypothesis that the phenotype underlies a parent-of-origin effect.

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E-P08.15

Rare microduplication in the 2p16.1p15 chromosomal region: implication on intellectual disability

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Introduction: Several patients with the 2p16.1p15 microdeletion syndrome have been reported. However, for microduplication in the 2p16.1p15 chromosomal region only one case has been so far described, which shows milder clinical features compared to those attributed to 2p16.1p15 microdeletion syndrome. In this report we describe a 2-year-old child suffering of mild intellectual disability and sharing clinical and genetic characteristics with the microduplication case previously reported. **Materials and Methods:** Array-CGH assay was carried out (Agilent CGH array 180k) following the manufacturer's instructions on a DNA sample isolated from peripheral blood cells. Quantitative PCR was performed using 7300 Real Time PCR System (Applied BioSystems) on the samples of our proband and his parents to determine the inheritance of chromosomal aberration. **Results:** Array-CGH analysis revealed the presence of a interstitial 2p16.1p15 microduplication encompassing a 1,997,488 bp region. No other pathogenetic genomic imbalance was detected in the proband sample. In the microduplication are presented 12 RefSeq genes. Among these *BCL11A*, *PAPOLG*, *REL*, *PUS10*, *PEX13*, *USP34*, *XPO1*, *FAM161A* and *CCT4* are included in the OMIM database. *PEX13* and *FAM161A* are the only genes classified as morbid. In order to confirm the duplication, quantitative PCR was performed using proband and parent's specimens. This procedure validated the presence of the duplication only in the proband (*de novo*). **Conclusions:** Clinical features and genetic data appear to be shared between the two cases of microduplication 2p. This observation could support the idea that the

microduplication in 2p16.1p15 chromosomal region might represent a newly defined syndrome.

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Chromosomal microarray testing in patients with developmental delay/intellectual disability, autism spectrum disorders, and multiple congenital anomalies: A Korean multicenter study

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Introduction: The aim of this multicenter study was to demonstrate the usefulness of chromosomal microarray (CMA), as a first-tier diagnostic test for developmental delay (DD), intellectual disability (ID), autism spectrum disorders (ASD), and multiple congenital anomalies (MCA) with unexplained etiology in Korea. Materials and Methods: We undertook CMA in 377 patients with idiopathic DD/ID, ASD, or MCA and 64 probands' families (parents or siblings) recruited from four tertiary hospitals and conducted a retrospective chart review of all patients to quantify the proportion of cases where CMA results impacted management recommendations for clinical action. Results: A total of 68 patients (68/377, 18.0%) had clinically relevant results, either abnormal ($n = 36$) or variants of possible significance (VPS, $n = 32$). The diagnostic yields of CMA were significantly higher than those of banding cytogenetics (22/377, 5.8%, $P < 0.001$). Twenty four well known-diseases were detected; Prader-Willi/Angelman syndrome was the most common ($n = 4$) and followed by DMD, 7q11.23 duplication, 15q11-q13 duplication, 16p11.2 microdeletion, 17p13.3 duplication, and DiGeorge syndrome. The size of variants of abnormal and VPS cohorts ranged from 142Kb to 151 Mb (median, 3.2 Mb). For patients with clinical follow-up available, CMA results had management implications for 87.5% and 78.1% of patients with abnormal variants (28/32) and with the VPS (25/32), respectively. Clinical recommendations included medical referrals, diagnostic imaging, pharmacological treatment, and contraindications. Conclusions: Clinical application of CMA as a first-tier test improves diagnostic yields and

influences medical managements in patients with DD/ID, ASD, or MCA.

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E-P08.17

Mild phenotypic changes in a child with major duplication of the short arm on chromosome 9

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Chromosome 9 is frequently involved in variable chromosomal imbalances due to the breakpoint sites along the chromosome, mainly on 9p. Chromosomal rearrangements include deletions, duplications and translocations. Partial duplication of 9p occurs mainly on the terminal part of the chromosome and has clarified clinical presentation. However, duplication on the more centromeric part of the chromosome is rarely described. Most of the karyotype -to-phenotype studies show that the severity of the clinical presentation is concordant with the length of the duplicated region. We present a young infant who was referred for cytogenetic analysis due to the hypotonia and coarse facial appearance. Karyotype survey revealed partial duplication on chromosome 9p11–21. Parental karyotype was normal. Dysmorphic features include broad nose, up-slanted and narrow palpebrae, low-set ears, macrostomia, low-set ears. Examination of all organs and systems revealed only minor atrial septal defect that closed spontaneously. Follow-up of the psychomotor development was done, continuously with abilities on lower normal scale for the age. Partial duplication of chromosome 9p is one of the most frequent structural abnormalities with significant phenotypic similarity in described cases. Although the region that has been duplicated is large and includes more than 50 genes, clinical presentation in our patient is mild without any major congenital anomaly. We can conclude that there is no significant overexpression of genes in this region that can produce potentially harmful congenital abnormalities and profound intellectual disability.

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E-P08.18**Homozygous variant of PGAP1 as a cause of severe developmental delay and intellectual disability identified by Exome sequencing**

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Introduction: Next generation sequencing has revolutionized the whole scenario of medical sciences and detection rate has dramatically increased especially in cases with non-syndromic developmental delay and intellectual disability.

Material and methods: We report here a family; a young non-consanguineous couple with history of two spontaneous first trimester miscarriages, followed by birth of two females and one male offspring, who presented with developmental delay, intellectual disability and associated facial dysmorphism at the age of 12, 10 and 2 years respectively. Conventional karyotyping and various molecular modalities were utilized for investigations

Results: Exome sequencing showed presence of homozygous variation 2286 + 5 G > A in intron 23 of *PGAP1* gene. This variant is linked to autosomal recessive mental retardation-42 (MRT42). This was further confirmed by Sanger sequencing in all affected siblings and parents.

Conclusion: We report siblings with homozygous mutations in *PGAP1* and enlighten the emerging clinical phenotype of *PGAP1* related disease. Our study also emphasizes the utility of Clinical Exome as an ultimate diagnostic tool for non syndromic cases.

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E-P08.19**Recovery from Rapid deterioration in individuals with Down syndrome**

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A small percentage of adolescents and young adults with Down syndrome (DS) experience unexplained regression in behavior, activities of daily living. This acute regression, which known as “rapid deterioration” or “catatonia in Down syndrome,” is one of the main concerns among the families and clinicians. Since the etiology of this condition is unknown, no treatment regimens are available. Here we present the cases of five adolescents with DS who recovered from rapid deterioration within one year. Common features deterioration observed in these individuals were significant loss of speech, loss of interest, abrupt deterioration of activities of daily living, moving slowly, eating slowly, and talking to themselves frequently. Prior to onset, none had an autism spectrum disorder. Trigger episodes causing the deterioration were not apparent except in one patient who experienced parental divorce. One 17-year-old female patient recovered from the decline after 2 months of co-sleeping with her mother, two got restored after transferring to new school or work place, and two got restored by giving their special schedule in day-care centers.; however, since the other 11 individuals did not show any improvement, an effective management strategy remained unclear. Our cases suggest that although rapid deterioration in DS is not a rare phenomenon, recovery is difficult, but some cases demonstrate that this condition can be reversible on early recognition by caregivers and medical personnel. Further studies with more cases may aid in the identification of a treatment strategy as well as prevention of rapid deterioration in young adults with DS.

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E-P08.20**Microdeletions and duplications as genetic cause of neurodevelopmental disorders in Bulgarian patients**

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Copy number variations (CNVs) are frequent cause of neurodevelopmental disorders where intellectual disability (ID) and seizures are part of more complex phenotype. Non-recurrent genomic rearrangements often result from reparative mechanisms such as Non-Allelic Homologous Recombination (NAHR) during meiosis. In the present study we performed array Comparative Genomic Hybridization (aCGH) using Agilent Microarray Kit, 4 × 180 K in Bulgarian patients with comparable neurodevelopmental abnormalities such as epilepsy, intellectual disability, autistic and other neuropsychiatric features. A confirmation quantitative PCR of all aberrations was performed using SYBR Green qPCR technology and $\Delta\Delta Ct$ method. aCGH revealed three deletions and two duplications in 4 patients affecting different chromosomes. The duplications were with size 1.856 Mb and 0.687 Mb and affected 17q12 and 9q33.1 regions, respectively. They were found in youngsters with absence and generalized tonic-clonic seizures, mild ID, learning disability and behavior problems. In 4 years old girl with ID, ataxia, seizures and delayed speech two microdeletions affecting 1q36 and 16p13.3 were detected- regions known to be associated with microdeletion syndromes. Additionally, homozygous deletion covering the first two exons of *IMMP2L* gene was found in girl with similar clinical characteristics. Despite the overlapping phenotype observed in our patients it appears that it is due to different chromosome regions affected. Therefore, it is important to use more extensive genomic approaches, such as chromosomal microarray analysis in order to clarify the actual genetic causes of these complex conditions. The study was supported by MU444/2016 of MU- Sofia, DTK/67/2009 and DUNK 01-2/2009 of NSF, Ministry of Education and Science.

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E-P08.21**FISH and karyotyping reveal potential chromosome structure anomalies under pure microdeletion/microduplication identified by chromosome microarray and its implication in prenatal diagnosis**

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Introduction: Some pure segmental chromosome microdeletion or microduplication detected by chromosomal microarray may underline potential structure anomalies, which might be lead to high recurrent risk. So it is necessary to recognize potential structure anomalies for the family. **Materials and Methods:** We report 6 cases with intellectual disability and other anomalies, 4 cases were found with segmental duplication and deletion by chromosome microarray: 9q del/22q dup, 16p del/19q dup, 10q dup/del, 18p del/18q dup, other 2 cases with pure microdeletion: 13qter microdeletion, 3p13-14 microdeletion. **Results:** Combined with FISH test and karyotype analysis, the structure 'hide' in were all revealed for the 6 cases as der(9)t(9;22), der(16)(16;19), inv dup del (10q), inv dup 18q/del 18p, 13qs, der (3)ins(18;3). Among these unbalanced rearrangements, 3 were inherited from their healthy parents who carried balanced chromosome rearrangements, 3 were de novo. 4 families have been provided with prenatal diagnosis at second pregnancy. **Conclusions:** Some pure segmental chromosome microdeletion or microduplication detected by chromosomal microarray usually associated with chromosome structure anomalies. Chromosome microarray, combined with traditional karyotyping and FISH, was useful to discover the potential structure changes underline copy number variations, which could help provide accurate recurrence risk evaluation and allow guiding prenatal diagnosis or reproductive planning.

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E-P08.22**Chromosome Fragile Site FRAXA Visualization in Fragile X syndrome patients**

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Introduction: Fragile sites on chromosomes are elements of human karyotype and can be visible on metaphase chromosomes as breaks or restrictions. Two types of fragile sites can be found on chromosomes: Common fragile sites – part of normal chromosomes and Rare fragile sites – result of repeat expansion mutation. Rare fragile site FRAXA located on Xq27.3 and appears as a result of CGG repeat expansion on 5' promoter region of FMR1 gene. People with expanded CGG repeat in FMR1 promotes have Fragile X syndrome – most widespread reason for inheritable mental retardation. Standard method of fragility analysis is based on routine Giemsa staining of metaphase chromosome and microscopic screening for breaks and restrictions. Percent of counted fragile sites depends on microscope magnification, slides quality and accuracy of researcher. Results: We developed the method for FRAXA fragile sites detection using two colored fluorescence *in situ* hybridization with two BACs. One is BAC clone containing full length human FMR1 gene that was used as fragile site marker, another BAC clone contains GPR50 gene. First validation of the method was made on cell cultures derived from Fragile X syndrome patients with control on normal cell lines. Statistical analysis revealed significance of difference in signals patterns between affected and control cell lines. Patients blood samples were analyzed with using of the method. Funding: The study is supported by Russian Science Foundation Grant 15-15-10001.

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E-P08.23**Paternal transmission of a FMR1 full mutation allele**

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Introduction: Fragile X syndrome is the most common form of inherited intellectual disability and autism. The molecular defect is a CGG repeat expansion in the 5' untranslated region of the *FMR1* gene. Full mutation (FM) expansion from premutated alleles (PM) is only acquired via maternal meiosis. Paternal transmission always remains in the PM range, from both PM and FM fathers. We present paternal transmission of a *FMR1* PM-FM allele in a 16-year-old girl with a mild phenotype with learning difficulties and anxiety. Material and Methods: The *FMR1* CGG repeat expansion was molecularly characterized using the AmplideX® PCR/CE FMR1 Kit and AmplideX® mPCR FMR1 (Asuragen) in peripheral blood from the patient and her parents and in semen from the father. *FMR1* gene expression analysis was performed using qPCR. Results: The patient inherited a normal allele (30 CGG, 2 AGG) from her mother and a mosaic PM-FM non-methylated allele (117->200 CGG, no AGG) from her father. Normal or slightly increased *FMR1* mRNA levels were detected. The father showed an 88 CGG uninterrupted non-methylated allele in both blood and sperm cells. Conclusion: The patient inherited a PM allele from her father that expanded to a FM in some cells and remained unmethylated. A postzygotic event (somatic expansion) is the most plausible explanation for this paternal transmission. This case raises important questions such as whether prenatal diagnosis should be offered in cases in which the father is the *FMR1* PM carrier. Acknowledgements: ISCIII [(PI12/00879], FEDER CERCA Programme and AGAUR (2014 SGR603) CIBERER (ISCIII).

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E-P08.25**Detection of a de novo heterozygous missense variant in GRIN1 as a potential causative mutation in a girl with slight motor delay and stereotypic movements**

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Pathogenic mutations of the GRIN1 gene (previous names NMDAR1; GluN1), which encodes for the two GluN1 subunits of the NMDA receptor, have been

described as causative for early-onset epileptic encephalopathy with severe intellectual disability, speech delay, muscular hypotonia, movement disorders and seizures in the absence of specific dysmorphic features. In this case study we report on an unexpected phenotype associated with a de novo GRIN1 variant. A 13 months old girl presented with slight motor delay, stereotypic movements of the hands and head and absence seizures. A prominent forehead, antverted ears and a tent-shaped upper lip were the only dysmorphic features. MRI and EEG revealed no abnormalities. We performed chromosomal microarray with no pathogenic result and ruled out Rett syndrome via sequencing and MLPA of the MECP2 gene. With clinical exome sequencing (TruSight One, Illumina) we revealed a so far unreported heterozygous variant c.2552 T>C (p. Leu851Pro) (NM_001185090.1), located in the important fourth (M4) transmembrane domain of the glutamate ionotropic receptor NMDA type subunit 1 (GRIN1). All eight used gene prediction programs classified the variant as pathogenic. Neither the mother nor the father of the girl are carriers for this variant. The de novo GRIN1 alteration c.2552 T>C (p.Leu851Pro) represents a potential causative mutation responsible for the girl's clinical phenotype. Her future development needs to be followed up. In conclusion our data indicate that a GRIN1 associated disease could be an important differential diagnosis of patients with intellectual disability, movement disorders and seizures.

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E-P08.26 A new homozygous mutation in the C12orf4 gene causing ID and speech delay

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The C12orf4-gene was recently identified as a new gene for a non-syndromic autosomal recessive intellectual disability disorder with only 9 patients described so far. Utilizing whole exome sequencing (WES) we identified a hitherto undescribed homozygous mutation in the C12orf4 gene in a 7 year old Portuguese girl born from consanguineous mating. Phenotypic features were subtle with deep set eyes, synophrys, pointed nasal tip, retrognathia, truncal adipositas and muscular hypotonia, and were similar

to those in the published cases. Her psychomotor development was moderately delayed, with independent walking at 2 years and receptive and expressive speech delay. C12orf4 is located on 12p13.3 and encodes for a 552 aminoacid protein. It is expressed in various tissues including the cerebral cortex and the cerebellum and plays a role in mast cell activation. Defects in the mast cell activations have been involved in abnormal function of the nervous system, but functional studies are needed to explore the molecular mechanisms underlying the pathophysiology of this newly identified intellectual disability disorder.

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E-P08.27

Neuropathological and clinical characterization of two sibling with TBCK deficiency

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Introduction: Biallelic *TBCK* mutations have been associated with severe infancy-onset disease characterized by hypotonia, psychomotor retardation, and characteristic facies (IHPRF3). The disease course and clinical manifestations are variable with survival over more than two decades and reportedly characteristic features such as dysmorphic facies and an elongated and thickened tongue being rather subtle findings. **Materials and Methods:** Patients with intellectual disability (ID) and movement disorders, in whom genomic imbalances and fragile X syndrome had been excluded, were screened for exonic variants using exome sequencing. **Results:** In two female siblings born to consanguineous healthy parents, we identified a previously unreported stop mutation in *TBCK* (c.304 C>T, p.Gln102*) in a homozygous state. Both girls presented with extreme hypotonia and deceased aged 7 and 11 years. Neuropathological showed brain atrophy, demyelination, wide-spread nerve cell losses, and neuronal inclusions initially suggestive of a differential diagnosis of "lysosomal storage disease", were found. **Conclusion:** The investigation of these patients whose clinical phenotype was also described by Bhoj et al in 2016 extended the spectrum of *TBCK* mutations in IHPFR3 as a relatively new

neurodegenerative condition which is difficult to diagnose at the clinical level. As the first neuropathological report in IHPFR3, these patients showed massive, relatively unspecific neurodegeneration, except for the occurrence of a carbohydrate-positive substrate. Further studies may elucidate the nature of this substance and link it to metabolic steps controlled by TBCK.

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E-P08.29

A novel *KDM6B* gene *de novo* mutation in two siblings with a variable neurocognitive phenotype

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Introduction: The clinical- and genetic heterogeneity of neurocognitive disorders (NCD) poses a significant diagnostic challenge. Targeted NGS comprising exonic regions with disease-causing variation (hence clinical exome sequencing; CES) has become a first line diagnostic- and research tool in such instances. Material and Methods: Two siblings (male 22- and female 10 years) with NCD were analyzed. Both suffered from speech delay, intellectual disability, dysmorphic features and some autistic traits. The girl is severely affected and has unsteady gait. Following aCGH CES was used in order to elucidate the underlying genetic causes of their unclear and clinically variable NCD. Results: A novel nonsense mutation in *KDM6B* gene (17p13.1) was found in both of the siblings by CES. In addition, family analysis confirmed that this pathogenic variant occurred *de-novo* in both siblings, indicating likely germline mosaicism in one of their parents. Conclusions: *KDM6B* is H3K27 demethylase involved in transcription of HOX genes during embryonic development, similarly to *KDM6A* associated with Kabuki syndrome 2. Due to the close function of both genes, variable expressivity of phenotypes associated with pathogenic variation in *KDM6B* could be expected. According to the published literature we have identified a third "mutation" in *KDM6B* linked to the development of NCD. Our findings suggest that *KDM6B* could be another candidate gene in intellectual disability and thus should warrant further studies in this regard. Supported by 00064203, OPPK CZ.2.16/3.1.00/24022 and NF-CZ11-PDP-3-003-2014.

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E-P08.30

Elevated expression and amplification of rRNA genes in a mentally retarded child with 13p + abnormal chromosome: a familial case study

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Introduction: A cytogenetic and molecular genetic study of the family with a male child who had mental retardation and autistic features revealed an abnormal chromosome 13 bearing an enlarged p-arm with amplified ribosomal DNA (rDNA) in a boy and his father. Study and results: Cytogenetic analysis using standard G-banding and FISH with labeled rDNA probes revealed an abnormal chromosome 13 with an enlarged p-arm due to rDNA amplification in a male child, who had clinically confirmed mental retardation and an autistic behavior. This chromosome is evidently inherited from the father, who has morphologically the same chromosome, but is healthy. The karyotype of the mother was normal. Ag-NOR staining showed brightly stained large whole-p-arm nucleolus organizer regions (NORs) in a child and normal-sized NORs in his father with 13p + -NOR-amount mosaicism. qRT-PCR with specific primers showed highly increased levels of 18 S, 28 S and 5,8 S ribosomal RNA (rRNA) in the patient's blood samples compared to a normal healthy control donor. Both patient's father and mother had no elevated levels of rRNAs expression. Conclusions: In this case rRNA level seems to correlate with mental retardation in familial individuals with 13p + . Our findings of rRNA overexpression in a patient with mental retardation and his parents may show a possible link between the karyotype (p-arm enlargement due to rDNA amplification), rDNA functionality (rRNA overexpression), functional changes in the brain and mental retardation. The study is supported by Russian Science Foundation Grant 15-15-10001.

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E-P08.31

Homozygous novel variant in *MUT* in a patient with intellectual disability without metabolic derangement

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 Alikaşifoğlu**

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Introduction: Homozygous mutations in *MUT*, encoding for methylmalonyl-CoA mutase lead to methylmalonic aciduria, characterized by elevated levels of methylmalonic acid in blood and urine. Patients present with recurrent attacks of metabolic acidosis, vomiting, dehydration, and lethargy, eventually leading to failure to thrive and developmental delay. Clinical features display a spectrum based on the frequency and severity of metabolic attacks and acute complications. Some mildly affected patients with undetected attacks may present later with isolated intellectual disability (ID). **Method and Results:** Homozygous novel Val489Glu variant in *MUT* was detected by whole-exome sequencing in a 15-year-old boy with severe ID. He had hand stereotypies, irregular sleep patterns, and attacks of crying. He never had metabolic attacks or seizures. Anthropometric measures were at 50th centile. He had an elongated face with a high forehead and prominent ears. He was very active, he lacked eye contact and resisted physical examination. Audiological exam, cranial imaging, and metabolic tests (blood and urine amino acids, urine organic acids, blood acylcarnitine profile) were all normal. Fragile X, karyotype and copy number analyses were also normal. On detection of Val489Glu variant metabolic tests were repeated and all were normal. **Conclusion:** Methylmalonic aciduria diagnosis requires presence of excess metabolites in blood and urine. Although intellectually unaffected patients with excess metabolites in body fluids are known, ID without metabolic derangement was reported only once in 2 patients. This novel variant in a highly-conserved residue caused no phenotype despite being predicted as pathogenic with *in silico* prediction programs.

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E-P08.33

Microduplication 22q11.2 in two related cases: Atypical LCR22B-G Duplication

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A cluster of eight low-copy repeats (LCRs), LCR22A-H, in chromosome 22q11.21 region mediate meiotic non-allelic homologous recombination, resulting in deletions or duplications of various sizes and intervals. The DiGeorge/velocardiofacial syndrome (DGS/VCFS) is the most common microdeletion affecting proximal LCR22A-D interval. It is characterized by a spectrum of clinical abnormalities affecting multiple systems: cardiovascular, neurological, psychiatric and immune. Reciprocal proximal 22q11.2 microduplication is also detected with variable manifestation of intellectual disability, behavioral problems and hypotonia. A very few proximal 22q11.2 microduplication cases share some features with DGS/VCFS: heart defects, velopharyngeal insufficiency and urogenital abnormalities. Microduplications in the distal region of 22q11, involving LCRs D-H are also reported with variable size and phenotypic features such as developmental delay, speech disturbances, aggressive behavior, hearing loss. Here we report two male patients, maternally related first degree cousins with developmental delay, behavioral problems and similar dysmorphism: lop ears, narrow palpebrae, broad nasal bridge and tip. One of them had congenital glaucoma, and the other one minor cardiac defect. MLPA analysis revealed the same duplication in 22q11.2 region with the minimal duplication size of 3.4 Mb, with proximal breakpoint at LCR22-B and distal at LCR22-G. According to UCSC and dbVar database this is the first reported duplication with breakpoints in LCR22B-G 22q11.2 region. MLPA analysis of apparently unaffected parents did not detect the duplication. The presence of the LCR22B-G 22q11.2 microduplication in two cousins, who also have an aunt with severe mental retardation, suggests presence of balanced 22q rearrangement in this family.

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E-P08.34**European Network on Brain Malformations Neuro-MIG (COST Action CA16118)****G. Mancini on behalf of Neuro-MIG**

ErasmusMC, Rotterdam, Netherlands

Among congenital brain disorders, malformations of cortical development (MCD) are rare neurodevelopmental disorders, often resulting from genetic mutations. The emergence of novel neuroimaging and genomic technologies potentially challenges scientists and clinicians of efficiently interpreting and translating these data for the benefit of patients. In Europe, expertise on MCD is very fragmented and confined to personal interest of a few experts and basic scientists studying cortical development are not always connected with clinicians. The Neuro-MIG consortium, supported by the COST Action will, for the first time, bring together clinicians and researchers in the field of brain malformations, to create the interdisciplinary, pan-European Network Neuro-MIG, advancing the understanding of MCD pathophysiology and translating this knowledge to improve the diagnostic and clinical management. This Action will harmonise MCD classification, based on the advances in genetics and neuroimaging, develop guidelines for clinical management, create best practice diagnostic pathways, coordinate databases from different countries to utilize them for collective research initiatives aimed at developing appropriate therapies, identify common pathophysiological mechanisms, educate young clinicians and scientists and stimulate translational and transnational exchange. Among the planned activities a web-based platform for case discussion will be provided. This Action will join forces of MCD experts to reduce health care costs and increase the quality of life of the affected individuals and their families. The Action is open for participation in one of the workgroups until October 2017: for those interested check eligibility (www.Cost.eu, Action CA16118) and contact the Action Chair (G. Mancini, g.mancini@erasmusmc.nl).

G.M. Mancini on behalf of Neuro-MIG: None.**E-P08.35****Intragenic deletion in the NPAS3 gene identified in a patient with mild intellectual disability and autism spectrum disorder****M. Smyk, K. Sobecka, M. Bartnik-Glaska, N. Bezniakow, J. Castaneda, B. A. Nowakowska**

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The *NPAS3* gene encodes a transcription factor of the basic helix-loop-helix family involved in central nervous system development and adult neurogenesis. Disruption of *NPAS3* has been described in a mother and daughter diagnosed with schizophrenia and mild learning disability. Association studies have linked single nucleotide polymorphisms in *NPAS3* with increased risk of schizophrenia, major depression and bipolar disorder. *Npas3* knockout mice show a deficit in adult hippocampal neurogenesis, aberrations in synaptic transmissions and display a range of behavioral phenotypes. *NPAS3* is a locus with an exceptionally high number of human specific accelerated regulatory elements (HAEs), i.e., non-coding regions highly conserved during mammalian evolution but accumulating sequence changes in the lineage leading to humans after human-chimpanzee split. We present a 7-year-old boy diagnosed with mild intellectual disability, autism spectrum disorder, discrete facial dysmorphism and clinodactyly of 5th fingers. Thus far only two *NPAS3* deletions have been described in patients with mental retardation or cognitive decline and psychosis. ACGH analysis in the patient using CytoSure ISCA, OGT detected a 392 kb deletion at chromosome 14q13.1 encompassing exons 4 and 5 of the *NPAS3* gene. Parental aCGH analysis revealed that the deletion occurred *de novo*. The deletion disrupts the PAS (period, aryl hydrocarbon receptor, single minded) domain required for dimerization and removes HAEs: HCNS96, 2xHAR142, HAR89, which act as transcriptional enhancers during development, particularly within the nervous system. This case further supports the causative role of *NPAS3* alteration in neurodevelopmental disorders. This work was granted from National Science Centre (OPUS 2015/17/B/NZ5/01357 to BN)

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E-P08.36**Novel frameshift mutation in exon 8 (c.1112delT) of NF1X gen in a patient with Malan syndrome**

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Sotos Syndrome (SS[MIM #117550*]) is an overgrowth syndrome characterized by tall stature and/or macrocephaly, distinctive facial appearance and intellectual disability. In

the 90% of SS patients, variations on NSD1 gene causing haploinsufficiency were identified. Among the remaining, point mutations or small intragenic deletion in the Nuclear Factor I-X gene (NFX1) were described as Sotos-like phenotype (Sotos 2) and recently proposed as Malan Syndrome (MS[MIM#614753]). NFX1 is also implicated in the Marshall-Smith syndrome (MSS[MIM#602535]). It was postulated that frameshift and splice site mutations around exons 6–8 of NFX1 gene may escape nonsense-mediated RNA decay, and therefore exert dominant-negative effect with strong deleterious consequences leading to the MSS. We present a 30 months old female referred to us in order to study her developmental delay and macrocephaly. The birthweight was in 50th centile, the length/height in 75th centile and OFC in 90th centile. She presented frontal bossing, hypertelorism, slightly downslating palpebral fissures, divergent strabismus, everted lower lip, and prominent chin; also pectus excavatum, several CAL spots and slight generalized hyperlaxity. No other relevant clinical findings were found. No pathological variations were identified in the aCGH (qChip^R, 60 K), MLPA and sequencing of NSD1 gene. Instead, a de novo frameshift variation in exon 8 (c.1112delT, p.Phe 371Serfs*30) was identified in the NFX1 gene. Variations in exon 8 are described in MSS patients but in our case, the phenotype is likely compatible with Malan syndrome. Further analyses on RNA are in progress in order to determine the effect of this new variation.

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E-P08.37

Further delineation of the TBL1XR1 molecular and phenotypic spectrum

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De novo mutations in *TBL1XR1* gene have been implicated in autism spectrum disorder, intellectual disability. *TBL1XR1* encodes transducin b-like 1 × -linked receptor 1 playing a role in transcription mediated by nuclear receptors. Recently, a specific *TBL1XR1* mutation, c.1337 A > C (p.Tyr446Cys) was described as the cause of Pierpont syndrome, a condition with a characteristic facies, short stature, hearing loss, developmental delay and distinctive palmar and plantar fat pads. We report here a follow-up of

two unrelated patients with *TBL1XR1* mutations. The patient 1 with de novo c.1331 C > T (p.Pro444Leu) mutation had psychomotor retardation, learning disabilities without intellectual disability, mild dysmorphism and Tourette syndrome. The patient 2 with de novo c.734 A > G (p.Tyr245Cys) mutation had psychomotor retardation, intellectual disability, epilepsy, obsessive-compulsive disorder, short stature, mild dysmorphism with short 4th and 5th metatarsals. The mutation of patient 1 is located near the specific mutation of Pierpont syndrome but he had no share the distinctive features seen in Pierpont syndrome. The mutation of patient 2 have been already described by Armour et al. in a patient with similar clinical phenotype. He had moderate cognitive impairment, progressive spasticity, short stature, Tourette syndrome, and short metacarpals, phalanges and toes. The detailed comparison of these patients with the earlier described cases in literature contribute to further delineate the clinical spectrum of *TBL1XR1* mutations.

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E-P08.38

Novel TBR1 frame shift mutation in a boy with motor, speech and cognitive developmental delay and bifrontal pachygryria polymicrogyria

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Exome sequencing studies have identified de novo loss-of-function variants in TBR1-gene in individuals with autism spectrum disorders, intellect disability and growth retardation. The number of patients with intellect disability is still rare and there are no reports about cortical changes and resulting symptoms in patients with TBR1-gene mutations. Here we describe the phenotype of a 2 year old boy with motor, speech and cognitive developmental delay, however normal growth and good communicative abilities, who showed bifrontal pachygryria/polymicrogyria in MRI and a novel frame shift mutation c.1588_1594dup, p.Thr532Argfs*144 in exon 6 of the TBR1-gene. No other mutations have been identified that could be causative for the cortical alterations. Thus, the symptoms of our patient extend the phenotypic spectrum of TBR1 mutation carriers. This study was funded by a grant of MMB.

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E-P08.40

SOX10 mutations in Waardenburg syndrome: clinical and molecular characterization of three new patients

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Waardenburg syndrome (WS) is a rare disorder characterized by sensorineural deafness and pigmentation defects. It is classified into four subtypes (WS1 to WS4) according to clinical and genetic background. Absence of clinical features characterizes WS2, while association with Hirschprung disease or neurological involvement defines WS4 and WS neurologic variant, respectively. WS is genetically heterogeneous, with six genes already identified including SOX10. We report on three new unrelated patients with WS2 and mutations in SOX10. Clinical description: All three patients presented with congenital sensorineural deafness. Age at diagnosis ranged from 2 to 5 years. Pregnancy course and delivery were normal in all three. No relevant family history, except for patient 2, with presumably affected father. All of them presented with variable degree of developmental delay. Brain MRI revealed hypoplasia or aplasia of semicircular canals in 2/3. Cutaneous hypopigmented patches and bright blue irides were present in 2/3. Retinal hypopigmentation in 2/3. None had Hirschprung disease. A pathogenic heterozygous SOX10 mutation was detected in all three, two of them previously undescribed truncating mutations. 2 de novo, one presumably inherited. One of the patients also had a maternally inherited DDX3X frame-shift mutation that modifies his neurologic phenotype. Conclusions: We report on three new patients with SOX10 pathogenic mutations, two of them not previously described, and highlight marked variable expressivity. SOX10 mutation analysis should be considered in patients with developmental delay and congenital sensorineural hearing loss even when no evident

pigmentary anomalies are present, and should be searched for, including assessment of ocular fundus.

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X - inactivation pattern in girls with idiopathic neurodevelopmental features

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Introduction: Gene compensation between XX females and XY males comes usually with a random inactivation of one of parental X chromosomes in the female embryo (X chromosome inactivation, XCI, a ratio of 1:1 between both X chromosomes). Deviation from this ratio is known as nonrandom inactivation of the X chromosome (NXCI). The XCI was analyzed in girls with idiopathic neurodevelopmental features (IND). **Materials and Methods:** We examined DNA isolated from blood of 98 girls with IND (aged 0–21), and 115 control girls (aged 0–16). Allele ratios of microsatellite polymorphism from human androgen receptor gene were used to determine XCI status. **Results:** XCI status from girls with IND was found to be nonrandom in 60.3% (44 out of 73 heterozygotes), whereas control girls showed NXCI in 44.2% (42 out of 95), which is a statistically significant difference ($p = 0.028$). In subgroups of girls with IND following NXCI percentages were observed: in intellectual disabilities (ID) subgroup 75% ($p = 0.082$; 6 out of 8), in subgroup with dysmorphic signs 62.8% ($p = 0.046$; 22 out of 35) and in subgroup with developmental delay (DD) 53.3% ($p = 0.329$; 16 out of 30). **Conclusions:** Our study identify significant differences in XCI pattern in girls with IND when compared with healthy control girls. We concluded that the determination of XCI may help interpret the role and effect of any genetic alterations on chromosome X detected in girls with IND.

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E-P08.42

Skewed X-inactivation in females with male children

affected by X-linked intellectual disability as a cellular marker for genetic counselling

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Introduction: The X chromosome inactivation is an epigenetic event that occurs randomly in every somatic cell of eutherian females to achieve gene dose compensation. Since skewed X inactivation may appear for a variety of reasons, including chromosomal abnormalities and mutations, we aim to verify if the high incidence in females with male children affected by X-linked intellectual disability is observable in a Brazilian population and if this phenomenon may be used as a cellular marker for genetic counselling. Methods: The control group is composed of 120 Brazilian females with a male child, both with no intellectual disability. The case group consists in 39 not intellectually disabled Brazilian females with male child affected by X-linked intellectual disability. To establish the X inactivation pattern, a STR in the Androgen Receptor (AR) was genotyped using the AR assay. Results: The control group presented out of 120, four women with skewed ratio. However, three of them had family background of cancer, showing skewing as an indicative for cancer susceptibility. In the case group, ten presented partial skewed ratio and five had extreme skewing. Conclusions: It was possible to verify a higher incidence of skewed X inactivation in women with affected male child, as shown by the literature. In future studies, we will further examine the association of the skewing with pathogenic CNVs in the X chromosome using microarray analysis, and hence finding the use of this assay in genetic counselling. Acknowledgments: CNPq and FAP-DF.

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E-P08.43

ACSL4 intragenic deletion in a boy with complex phenotype

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Introduction: X-linked intellectual disability represents an extremely heterogeneous condition for which more than 100 genes have been described. A X-linked mental retardation-63 syndrome caused by mutation in the ACSL4 (300157) gene and characterized by mild to severe mental retardation, behavioral manifestations, microcephaly and language impairment (in some patients), have been described. Materials and methods: In this paper we report on a 10 years old boy with a complex phenotype, including severe intellectual disability (IQ 25), severe speech delay, microcephaly, severe growth delay, delayed bone age, and dysmorphic features. His cerebral MRI and hormonal screening were normal. NGS of 4813-gene panel (TruSight One, Mendeliome) and Sanger-sequencing of exon 12–14 flanking regions of ACSL4 gene was performed. Results: Quantitative analyses of the NGS data identified a hemizygous intragenic deletion in the ACSL4 gene, spanning part of exon 12–exon 14 with a size of 5049 bp: seq [GRCh37/hg19]Xq22.3(108,903,631–108,908,680). Only four pathogenic point mutations in patients with intellectual disability have been reported so far (Meloni et al., 2002; Longo et al., 2003; Yonath et al., 2011). A deletion involving only ACSL4 gene or an intragenic deletion has not been described previously. Conclusion: The phenotype of our patient is more severe than other cases reported before. Additional experience is needed in order to better delineate the relationship between ACSL4 intragenic deletion and phenotype severity.

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E-P09 Neurogenetic and psychiatric disorders

E-P09.01

Clinical and genetic analysis of four Czech families with 15q13.3 microdeletion syndrome

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The 15q13.3 microdeletion is a genetic disorder characterized by a range of neurodevelopmental disorders,

including seizures, cognitive and social impairments, autism, and schizophrenia. 15% of cases are *de novo* and 85% are inherited. The phenotype varies in spectrum and severity owing to incomplete penetrance or variable expressivity. The 15q13.3 microdeletion is typically 1,6 Mb, harboring at least seven genes. Haploinsufficiency of *CHRNA7* is causative for the majority of neurodevelopmental phenotypes in the 15q13.3 microdeletion syndrome. We present clinical, cytogenetic and molecular analysis of four cases with the 15q13.3 microdeletion. All findings were confirmed by FISH. In the first case, array CGH revealed a 1,86 Mb maternally inherited microdeletion in a boy with language and psychomotor delay, moderate intellectual disability, hyperactivity, and aggressive behavior. A 2,02 Mb maternally inherited microdeletion at 15q13.2q13.3 was found in the second case. The boy manifested developmental and language delay, ADHD, and visual impairment. In these two cases, mothers were asymptomatic carriers of the microdeletion. The third case presents a girl with language and psychomotor delay, impulsive and aggressive behavior, autism, and hypotonia. Here we detected a 1,69 Mb microdeletion in the 15q13.2q13.3 region and also a 506 kb intragenic microduplication of *CNTN4* gene in the 3p26.3p26.2 region using array CGH. The *CNTN4* gene is considered as a candidate gene in ASD. The proband's mildly affected mother showed a 1,56 Mb microdeletion at 15q13.2q13.3. The last case represents a boy with a 1,47 Mb microdeletion of 15q13.2q13.3 and clinical features: language and psychomotor delay, and ADHD.

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E-P09.02

9q21.13 microdeletion syndrome masquerading as Angelman syndrome

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9q21.13 microdeletion syndrome was discovered after using chromosomal microarray techniques to identify chromosomal abnormalities. There are several candidate genes in this region for mental retardation and epilepsy. The main characteristics include intellectual disability, speech delay, epilepsy and specific facial features. Our patient was an 8-year-old boy with the original diagnosis of Angelman syndrome. His prenatal and perinatal periods were uneventful. His development seemed normal until the age of 8 months, at which point it stalled after he developed seizures. His epilepsy was difficult to control, requiring several antiepileptic medications. When he was 8-year-old, he demonstrated severe intellectual disability by unstable walking with wide-based gait and uplifted arms, absence of meaningful word production, inability to communicate, and autistic behavior. Physical examination revealed failure to thrive, microcephaly, bitemporal narrowing, thick eyebrows, long and smooth philtrum, thin upper lip, borderline low set ears, and micropenis. Brain MRI showed only pituitary hypoplasia. Angelman syndrome was diagnosed based on his clinical features and epilepsy, although the chromosome study, FISH for 15q11.2, *SNRPN* methylation test, and *MECP2* sequencing analysis, which were also negative, given 20% of the cases have a diagnosis with the negative DNA methylation test. Interestingly, the chromosomal microarray revealed *de novo* deletion of 7 Mb on the long arm of chromosome 9 (9q21.11–9q21.2). Therefore, our patient was re-diagnosed as having 9q21.13 microdeletion syndrome. In summary, Angelman syndrome patients with negative test results should be considered for further investigations, given Angelman syndrome may not be the final definite diagnosis.

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Autosomal dominant cerebellar ataxia, deafness and narcolepsy (ADCA-DN): report of the first Central European case with pathogenic variation in the *DNMT1* gene

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Introduction: Autosomal dominant cerebellar ataxia, deafness, and narcolepsy (ADCA-DN) is a rare adult onset neurologic disorder caused by heterozygous pathogenic variation (PV) in *DNMT1* located on chromosome 19p13. ADCA-DN shows clinical overlap with hereditary sensory and autonomic neuropathy type 1 with dementia and deafness (HSAN1E) and is associated with PV in the same gene, i.e. *DNMT1*. To date, eight ADCA-DN families have been reported (coming from e.g. Italy, USA, Sweden, Canada) with four distinct underlying pathogenic variants in *DNMT1*. Thus far, no cases with PV in Central European patients with ADCA-DN have been reported. Methods: Following complex neurologic and clinical genetic examination bidirectional Sanger DNA sequencing of exons 20–22 in *DNMT1* was performed on the 3130xl platform (ThermoFisher Scientific, USA). Results: We found a previously described missense variant p.Ala570Val in *DNMT1* in a 62 year old male patient who has been suffering from narcolepsy since the age of 41. He showed first signs of hearing impairment at the age of 52, had first signs of ataxia at 55 and became deaf at 56. Similar progressive development had been documented in five other family members from within three consecutive generations. Discussion: The p.Ala570Val PV was previously detected in two Italian families and one family from USA and Canada, respectively. Thus this is the first report of a *DNMT1* gene PV in a Central European patient with ADCA-DN. Supported by 00064203, OPPK CZ.2.16/3.1.00/24022 and NF-CZ11-PDP-3-2014.

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“ADHD Moves”, a novel network focussing on Mendelian subtypes and endophenotypes of attention deficit hyperactivity disorder (ADHD)

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Traits underlying “complex” inheritance (i.e., multiple genes and environmental factors contributing to the phenotype), such as a variety of psychological and psychiatric disorders, depend largely on generalized therapies, since biological markers are rare or absent. Accordingly, research aiming at the identification of genetic markers relevant for

diagnosis or therapy of attention deficit hyperactivity disorder (ADHD) was not successful so far. ADHD is the most common mental disorder in children and adolescents, and can persist into adulthood. It has a high heritability of 75% - 91%, which indicates a strong genetic influence. Intense research by large international consortia focuses on ADHD as a complex disorder. Thousands of samples of affected and non-affected subjects were genotyped in hypothesis-free genome-wide association studies (GWAS). One of the biggest of those studies conducted by Elia and coworkers in 2011 identified deletions and duplications of genes coding for glutamate receptors in some ADHD patients and their siblings. Thus, subtypes of ADHD following a Mendelian pattern of inheritance could be assumed. Only a small number of publications on linkage analyses of large multiplex families with ADHD (Lin et al., 2013, Vegt et al., 2010; Amin et al., 2009, Romanos et al., 2008, Arcos-Burgos et Al., 2004) exist to date. In our newly founded “ADHD MoveS” network, geneticists, psychologists, physicians and bioinformaticians will continue this promising line of research investigating pedigrees with an ADHD phenotype, and additionally apply genetic, psychometrically measurable and stable cognitive and biological parameters, so called endophenotypes.

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E-P09.06

Report of an ALS case associated with a new mutation in the TARDBP gene

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Introduction: Starting in early 2008, dominant mutations in TARDBP gene have been reported by several groups as a primary cause of ALS. To date, over 50 mutations of TARDBP have been described not only in SOD1-negative fALS cases but also in sALS cases. Materials and methods: The patient, a 80-year-old woman, was referred with a history of progressive weakness of the right leg. Her family history was negative. After an extensive diagnostic workup, a diagnosis of ALS, spinal onset, was made. Mutation screening in ALS linked genes was performed by bi-directional Sanger sequencing. Resources PolyPhen2, SIFT, Panther, were used for in silico characterization of mutant protein. The progression rate (Δ FS) gave a value of 4.2, indicating a rapidly progressing disease.

Ten months after diagnosis, she died because of a respiratory failure. Results: Molecular analysis identified a novel heterozygous substitution in exon 6 of TARDBP gene that leads to an amino acid change from serine to alanine at position 379 (p.S379A). Both PolyPhen2 and SIFT resources characterize p.S379A as benign while Panther classification system reports it with a SubPSEC of -1.00268, p = 0.11948. Conclusion: We describe an apparently sALS patient with a new heterozygous TARDBP mutation in the codon 379 of exon 6, characterized by a late onset and a rapid disease progression. This missense variation involves an evolutionary conserved aminoacid and lies within C-terminal Gly-rich domain, where the majority of ALS-related mutations of TARDBP described in patients with both fALS and sALS, have been clustered.

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E-P09.07 investigation of expressions of certain miRNAs in patients with Alzheimer's disease

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Introduction: Alzheimer's disease (AD) that is a progressive neurodegenerative disorder is the most common dementia among the elderly. It is pathologically characterized by extracellular amyloid plaques deposition, neurofibrillary tangles, synapse and neuronal loss. Recently, accumulating cases have demonstrated a direct link between microRNAs (miRNAs) and AD. miRNAs are single-stranded RNAs, approximately 22 nucleotides in length. miRNAs regulating post-transcriptionally gene silencing by partially bases pairing with the 3'UTR of target mRNAs play important roles in several pathways such as cell proliferation, differentiation, apoptosis and protein secretion. Materials and Methods: The blood samples derived from 84 AD patients and 67 control subjects were composed, to identify the expression levels of 6 candidate circulating miRNAs (hsa-miR-132-3p, hsa-miR-186-5p, hsa-miR-195-5p, hsa-miR-219a-5p, hsa-miR-3163 and hsa-miR-3613-3p) for AD. Total RNA isolation was performed by Trizol Reagent method from whole blood. The expression levels of selected miRNAs were analyzed by using comparative

$\Delta\Delta C_T$ method in Real-Time PCR. Data obtained from molecular analyze were statistically evaluated. Results: It was identified that hsa-miR-186-5p were markedly downregulated in AD patients, while hsa-miR-3163 and hsa-miR-3613-3p were significantly upregulated in AD patients compared to controls ($p < 0.05$). However, significant differences in expression levels of hsa-miR-132-3p, hsa-miR-195-5p and hsa-miR-219a-5p were not found between groups. Conclusions: These data indicate that hsa-miR-186-5p, hsa-miR-3163 and hsa-miR-3613-3p may contribute to AD-related neurodegeneration. Altered expression of these miRNAs might enable prospective applications as biomarkers and candidates for molecular therapeutic targets.

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Association of polymorphism rs2278749 gene ARNTL with psychosocial factors and sleep disturbances in male population 25–44 years in Russia/Siberia

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Objective: to study the association of polymorphism rs2278749 gene ARNTL with some components of affective disorders and sleep disorders in the male population 25–44 years in Russia / Siberia (Novosibirsk). Methods: In 2014–2016 a random representative sample of the male population 25–44 years surveyed in one of Novosibirsk districts. Randomly selected 200 men had a mean age of 35.5 years who underwent psychosocial testing. Questionnaire “4-item Jenkins Sleep Questionnaire» is used. The men included in the study, studied the frequency distribution of genotypes of rs2278749 ARNTL gene. Results: It was found that the most common genotype rs2278749 ARNTL gene was homozygous C / C genotype - 74.9%, C / T genotype was at - 22.3%, 2.8% - genotype T / T. It was revealed that carriers of the genotype C / T more likely to experience serious conflicts in the family, more experienced their frustration, they often have disturbing dreams, and they wake up tired and exhausted, in addition, they often met the high level of the life of exhaustion, and they soon became frustrated. Carriers of genotype T / T often took the trouble “to heart” and were more punctual. On the other hand, the carriers C / C genotype were more hostile, were inclined not to trust anyone, almost “never” accept negative situations “close to the heart” and much less experienced

disturbing dreams. Conclusions: determined that the genotype C / T ARNTL gene associated with sleep disorders in the Siberian population.

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Putative role of *BAI1* gene in a patient with pervasive developmental disorder

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Introduction We present a youngster bearing a *de novo* translocation, encompassing two genes: *TSNARE1* and *BAI1*. *TSNARE1* polymorphisms have been associated with schizophrenia and bipolar susceptibility, while *BAI1* plays a role in synapse function and regulates spatial learning. **Patients and Methods** Healthy 11 year-old boy attending regular school. Pervasive developmental disorder was suspected at four, with severe ODD, and ADHD. Conventional and molecular karyotypes were performed. FISH experiments were completed using BAC clones for breakpoint's approach. Gene expression assays were done using Taqman probes. **Results** The patient's karyotype was 46,XY,t(8;17) (q24.3;q22)dn. aCGH revealed no genomic imbalances. FISH analysis showed that the CTD-2599D24 probe spanned the translocation breakpoint at 8q24.3, involving 2 genes (*TSNARE1* and *BAI1*). Breakpoint at 17q22 was delimited between RP11-293B16 and RP11-277L08 probes, with a gene encoding a non-characterized protein involved. Gene expression assays showed low *BAI1* gene expression in the proband, compared to controls. **Conclusion** We speculate on the potential pathogenicity of the genes contained in the 8q24.3 region that are likely to be disrupted by the translocation breakpoint, and responsible for the clinical features of our patient. *BAI1* gene is mainly expressed in the brain. Recently, *in vivo* studies suggest that *BAI1* regulates spatial learning and synaptic plasticity in the hippocampus. *BAI1* also interacts with *BAIAP2/IRSp53*, a gene with a potential role in autism and psychiatric disorders. Thus, we propose that the impairment of *BAI1* gene is responsible of our patient's behavioral phenotype. Further studies are ongoing to elucidate the exact breakpoint of the rearrangement.

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E-P09.11

Usefulness of oligonucleotide array CGH in clinical diagnostics of autism spectrum disorders

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Autism spectrum disorders (ASDs) are one of the most common groups of neurodevelopmental disorders with the prevalence of 1–2%. Genetic factors play an important role in the etiology of ASDs; it has been estimated that sub-microscopic copy number variants (CNVs) are the cause of ASDs in ~ 5–13% of patients. We elected to use aCGH to evaluate its efficacy for identification and characterization of CNVs in a cohort of 178 patients with ASDs and to identify novel ASDs genes. The analyses of the patients' genomes were performed using genome-wide oligonucleotide microarrays (180k; Agilent Technology, OGT) with an average resolution of 30 kpz. We identified 25 CNVs in 21 out of 178 (11.8%) patients. Pathogenic or likely pathogenic CNVs were detected in 12 (6.7%) patients, whereas CNVs of unknown clinical significance were found in 6.2% of patients with ASDs. All of the identified CNVs were sub-microscopic in size (between 75 kb and 4.54 Mb) and thus could not have been detected by standard karyotype analysis. Our study further confirmed the potential of aCGH in elucidating the etiology of ASDs, demonstrated by the identification of two novel genes: *ARHGAP24* and *SLC16A7* as pathogenic for ASDs and three genes: *SNX19*, *PIGN* and *HCN1* as candidate for ASDs. The work was supported by grant R13-0005-04/2008 and project 3942/E-215/S/2016 from the Polish Ministry of Science and Higher Education

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E-P09.12**Synaptosome-Associated Protein 25 (SNAP25) Gene Association Analysis Revealed Risk Variants for ASD, in Iranian Population****M. Safari¹, M. Taheri²**

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Autism spectrum disorder (ASD) is a common, complex neurological condition, affecting approximately 1% of people worldwide. Monogenic neurodevelopmental disorders which showed autistic behavior patterns have suggested synaptic dysfunction, as a key mechanism in the pathophysiology of ASD. Subsequently, genes involved in synaptic signaling have been investigated with a priority for candidate gene studies. A synaptosomal-associated protein 25(SNAP25) gene plays a crucial role in the central nervous system, contributing to exocytosis by targeting and fusion of vesicles to the cell membrane. Studies have shown a correlation between aberrant expression of the SNAP25 and a variety of brain diseases. Single nucleotide polymorphisms (SNPs) in this gene are associated with several psychiatric diseases, such as bipolar, schizophrenia, and attention-deficit/hyperactivity disorder. The aim of the present study was to investigate whether polymorphisms (rs3746544 and rs1051312) in the regulatory 3'-untranslated region (3'UTR) of the SNAP25 gene have an association with ASD in unrelated Iranian case (N = 524)-control (N = 472) samples. We observed robust association of the rs3746544 SNP and ASD patients, in both allele and haplotype-based analyses. Our results supported the previous observations and indicated a possible role for SNAP25 polymorphisms as susceptibility genetic factors involved in developing ASD.

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E-P09.13**A Novel Frameshift Mutation in CACNA1A Gene in a Turkish Patient with Episodic Ataxia Type 2****B. Gerik-Çelebi¹, H. Mavioğlu², S. Çam¹**

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Introduction: Episodic ataxia type 2 (EA2, OMIM#108500) is an autosomal dominant inherited

disorder characterized by ataxia, dysarthria, vertigo, nausea and weakness. EA2 is due to mutations in the calcium voltage-gated channel subunit alpha1A (CACNA1A) gene mapped to the 19p13.13 chromosomal location and plays a role in taking a calcium ion into the cell by a voltage-gated channel. **METHODS:** CACNA1A gene sequence analysis including all coding exons and exon-intron boundaries was performed. **RESULTS:** We examined 20-year-old female patient who has been suffering from attacks of ataxia, headache, vertigo and also muscle weakness which has been progressed increasingly since the age of 13. Cranial MRI was showed hyperintensities consistent with frontal subcortical demyelinating plaques in pericallosal, corpus callosum, periventricular white matter. Sequence analysis was revealed a heterozygous mutation in CACNA1A gene NM_001127222.1: c.2259_2260insCG; (p.A754Rfs*6) and c.561 G > A (p.T187T). While we examined both parents for the mutation, father was found heterozygous carrier and mother was not present. **CONCLUSION:** In this study a novel CACNA1A mutation was reported. According to In silico analysis softwares this mutation causes premature stop codon and therefore it could be the cause of disorder. Although the father was the carrier of the mutation, the absence of the clinical finding is suggesting the incomplete penetrance or expressivity difference. Also synonymous mutations might be considered for the cause of clinical findings by making changes in protein expression and function. Therefore the reason of the disorder in this patient could be both gene and synonym mutations.

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E-P09.15**Differential diagnostic of a rare disease in movement disorders: the chorea-acanthocytosis****K. Hadzsiev¹, M. Szöts², A. Fekete¹, Care4Rare Canada Consortium³, F. Nagy⁴, B. Melegh¹**

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Introduction: Neuroacanthocytosis is a heterogeneous group of rare diseases characterized by progressive neurodegeneration and red cell acanthocytosis. It can be divided into several subgroups, one of which is choreoacanthocytosis. Aim: We sought out to explore the genetic background of a Hungarian family with two affected brothers with adult onset progressive movement disorder

and psychoaffective symptoms. Method: Following traditional clinical and molecular genetic examinations available in Hungary, whole exome sequencing was performed in collaboration with Care4Rare Canada. Results: A homozygous nonsense variant c.3903 G > A (p.Trp1301*) in the *VPS13A* gene was identified, which has been previously reported as a pathogenic variant. Conclusions: This clinical presentation represents a rare form of movement disorder and highlights the usefulness of next generation sequencing technologies, specifically whole exome sequencing, in the diagnosis of rare disease patients.

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Copy number variations (CNVs) of Alzheimer's disease susceptibility genes in a pediatric clinical cohort

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Introduction Alzheimer's disease (AD) is the leading cause of neurodegeneration. Complex AD etiology suggests the contribution of both genetic and environmental factors. Nowadays, there are about 150 AD susceptibility genes. However, their role in AD pathogenesis is generally unclear.

Materials/methods We evaluated 150 AD susceptibility genes in a clinical cohort of 450 children with autism and intellectual disability, which were studied using SNP-array molecular karyotyping and an original bioinformatics technology (Iourov et al., 2014).

Results Unique CNVs of *APP*, *PSEN1*, *NOS3*, *HFE* were found in 5 patients (1%). These CNVs possibly

increase AD risk in adulthood. CNVs of *CRI* and *CLU* had a higher frequency (4.8%). Partial duplication of *CRI* and partial triplication of *CLU* were found in 12 and 10 individuals, respectively. Partial *CLU* loss was found in a patient. *CRI* and *CLU* CNVs were defined as benign.

Conclusions Studying AD susceptibility gene CNVs shows that at least two types of candidate genes for this disease exist. The first type encompasses genes affected by rare CNVs, which are likely to be involved in AD pathogenesis, whereas the second type encompasses genes affected by common CNVs, which are likely to be benign. Furthermore, the second type is probably considered as related to AD because of the common involvement in genomic variations producing false-positive results in genome-wide association studies. Supported by the Russian Science Foundation (project #14-15-00411) (clinical cohort analysis) and ERA.Net RUS Plus Programme (AD studies).

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E-P09.19

Apolipoprotein E major alleles in surgery outcome of pharmacoresistant patients with temporal epilepsy

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Introduction: The Apolipoprotein E (ApoE) is a glycoprotein that transports lipids, thus maintaining and repairing neurons. The codifying gene is located in the chromosome 19. Two single nucleotide polymorphisms lead to changes of two amino acid's positions resulting in three major alleles ε2, ε3 and ε4. Temporal location is the most common form of pharmacoresistant epilepsy and is usually treated by surgery. The objective is to know if Apo E major alleles are associated with the prognosis of pharmacoresistant surgery patients with temporal epilepsy. Materials and methods: We selected 63 pharmacoresistant epileptic patients (38 women and 25 men) according to ILAE's criteria with temporal location. We classified them in two groups based on the outcome of surgery using the Engel classification. The scales I (50) and II (1) refer to patients free or with rare seizures and III (7) and IV (5) to a worthwhile improvement or not after surgery. The genotype was determined with the LightCycler® Real-Time PCR System. We measured the association between each ApoE allele and the two Engel groups using Fisher's test (p-value < 0.05 statistically significant). Results: p = 0.036

Major alleles	Engel Epilepsy Surgery Outcome Scale I + scale II	Engel Epilepsy Surgery Outcome Scale III + scale IV
Apo E ε3	25 (92%)	2 (8%)
Apo E ε2	23 (77%)	7 (13%)
Apo E ε4	3 (50%)	3 (50%)

Conclusions: The results suggest that the ε4 major allele is associated to a lower success rate of surgery for pharmacoresistant epileptic patients with temporal location which might be confirmed by an extensive study. This study was financed by "Instituto de Salud Carlos III", PI12/02839, partially supported by FEDER.

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E-P09.20

Single-exon deletion in the *SCN2A* gene in a patient with profound epilepsy and developmental delay

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We present the second case in literature of a child with a single exon deletion in the *SCN2A* gene on chromosome 2q24.3 detected by array CGH. The deletion is 3.5 kb in size and encompasses the complete exon 14. The 5 years old boy was presented to the genetic counseling practice because of profound epilepsy. The parents have realized regression of psychomotor and speech development since the first epileptic seizure with status in the age of 1 ½ years and above all are concerned by the stereotypical movements and behavioral problems of their son. There are a few unspecific, phenotypical abnormalities such as broad face with frontal bossing and oral hypotonia - the growth parameters lying in the normal range. The two elder brothers as well as the parents themselves are healthy. A cousin of our patient shows a genetically proved blepharophimosis, ptosis, and epicanthus inversus syndrome. Segregation analysis of the parents by quantitative PCR proved the deletion to be *de novo*. To the best of our knowledge there is only one further patient in the literature carrying a exon 14 deletion in the *SCN2A* gene (Horvath et al, 2016) due to a frameshift mutation. The 10 years old male patient had early onset global developmental delay, intermittent ataxia, autism, muscular hypotonia, epileptic encephalopathy, and cerebral/cerebellar atrophy.

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E-P09.21

Identification of one novel mutation in GRN gene associated with frontotemporal dementia

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Mutations in progranulin gene (GRN) are a common cause of autosomal dominant frontotemporal degeneration syndromes and are associated with a wide phenotypic heterogeneity. The majority of genetic defects in GRN consists of loss-of-function mutations, causing haploinsufficiency. Herein, we present the case of a 67-year-old right-handed man with a 6-year history of gradually progressive behavioural disturbances with irritability and sometimes aggressiveness, social withdrawal and obsessive repetitive behaviors. In the family history, we found an uncle from the paternal line affected by dementia with behavioral disorders and progressive language difficulties. Considering the early onset and the positive family history, a genetic analysis was carried out, showing the presence of a novel heterozygous missense mutation c.53 C > T in the GRN gene. The heterozygous C to T transition resulted in a threonine (ACG) to methionine (ATG) substitution (p.Thr18Met). In our patient, we identified a GRN missense mutation that was predicted to be probably damaging by the PolyPhen-2 software (with a score of 0.995, sensitivity 0.68 and specificity 0.97), and deleterious by the SIFT software (with a score of 0.01). GRN c.53 C > T was not found in 150 control subjects, but was detected in the ExAC browser with a very low minor allele frequency ($MAF = 4.5 \times 10^{-5}$). Our findings suggesting that (1) rare coding variability in GRN may influence the susceptibility to FTD and (2) highlight the importance of genetic analysis also in sporadic forms of FTD. In conclusion, our result enlarges the spectrum of clinical phenotypes requiring genetic analysis in search of mutations of progranulin gene.

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E-P09.23**McLeod syndrome in an Italian patient**

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Introduction: McLeod syndrome (MLS) is a rare X-linked multisystem disorder caused by mutations in the XK gene, encoding the Kx antigen on red blood cells. Peculiar laboratory findings are acanthocytosis and McLeod blood-group phenotype. The disease includes CNS features similar to Huntington's disease and neuromuscular manifestations. Patient and methods: We report the case of a 52-year old patient with moderate dysarthria, scratching skin lesions, limb hypotonia and muscle wasting with reduced tendon reflexes, diffuse choreic movements with prominent orolingual dyskinesias and walk instability. He had a family history of chorea and seizures in the maternal grandfather, whilst both his parents and his sister did not show any neurological anomaly. Brain MRI revealed caudate atrophy. Electromyography and nerve conduction study suggested sensory-motor axonal polyneuropathy of lower limbs. Blood tests showed prominent CK elevation. We also observed the presence of acanthocytes. RBC immunochemical examination showed no Kpa/Kpb antigens and extremely reduced expression of Kell surface protein, consistent with "McLeod blood group phenotype". Genetic analysis was performed by direct sequencing of the three XK gene exons and the intron-exon boundaries. Results: Molecular screening of the XK gene revealed the hemizygous five-base deletion c.856_860delCTCTA, in exon 3. This mutation was previously referred as 938-942delCTCTA. Conclusions: This is the first Italian case of MLS caused by a small deletion in exon 3 of the XK gene. This deletion creates a premature stop codon. Although there is not a clear genotype-phenotype correlation, molecular characterization is the gold standard in diagnosis and further classification of the syndrome.

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E-P09.25**A novel NOTCH3 gene mutation in a Turkish family**

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Introduction: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a small vessel disease caused by mutations of the NOTCH3 gene, located on chromosome 19p13.12, characterized by relapsing strokes with neuropsychiatric symptoms and affects relatively young adults of both sexes, but it is rarely associated with autoimmunity. Methods: We present the clinical, immunologic and molecular genetic findings of 65-year-old female patients applied to our hospital with dizziness for 6 years and her asymptomatic 45-year-old son. DNA sequence analysis was performed in the study. Results: In addition to dizziness, she had headache and small joint aches. ANA elevation was detected as a rheumatological marker. The carotid doppler USG showed 8 mm plaque in internal carotid artery of the patient who had normal cranial MRI. The undefined protein-encoding Proline496Leucine (c.1487 C > T) heterozygous mutation on the exon 9 was detected. This mutation highly likely to be the cause of the disease in silico evaluation tools. In the evaluation of her family, it detected in her son too. Discussion: This is a rare description of the coexistence with autoimmunity in CADASIL patients.

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E-P09.27**Polygenic risk score of SERPINA6/SERPINA1 influences diurnal and stress-induced salivary cortisol in children**

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Introduction: Cortisol secretion is elevated by activation of the hypothalamic-pituitary-adrenocortical (HPA) axis in response to stress. A genome-wide association study by the CORtisol NETwork (CORNET) consortium identified common variants at genes *SERPINA6* (Serpin Family A Member 6)/ *SERPINA1* (Serpin Family A Member 1) influencing the morning plasma cortisol and its binding protein in adults. Here, we investigated effect of these SNPs on diurnal and stress-induced salivary cortisol levels in Finnish children's from Glycyrrhizin in Liquorice (GLAKU) birth cohort. **Methods:** Diurnal salivary cortisol (seven samples/day) and salivary cortisol during the Trier Social Stress Test for Children (TSST-C, seven samples) were measured in 186 children (50.5% girls; mean age = 8.09 years, SD = 0.31) with genome-wide genotyping. We extracted 6 SNPs ($r^2 < 0.7$) showing p-values $< 5 \times 10^{-8}$ in the CORNET meta-analyses and calculated weighted polygenic risk score (PRS). Association between PRS and cortisol measurements were analysed with mixed model and linear regression analyses using SPSS Statistics 24. **Results:** PRS associated with higher diurnal salivary cortisol at bedtime (p-values < 0.008 ; PRS*time interaction p < 0.000032); higher nadir and cortisol awakening AUC increment (p < 0.030); and cortisol maximum values upon awakening minus bedtime values (p < 0.011). In TSST, PRS associated with maximum cortisol after stress minus baseline values (p < 0.042 ; PRS*stress time interaction p < 0.009), and cortisol maximum slope (p < 0.043). Linear regression analyses with Bonferroni's correction threshold p-value (p < 0.0083) showed two SNPs rs7161521 (*SERPINA6*) and rs4900229 (*SERPINA1*) are significantly associated with cortisol single indices. **Conclusions:** Common variants in *SERPINA6/SERPINA1* may influence the diurnal salivary cortisol and cortisol reactivity in response to stress in children.

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E-P09.28

Single Nucleotide Polymorphisms in *Foxp3* gene are associated with increased risk of relapsing-remitting multiple sclerosis

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Single nucleotide polymorphisms in the *FOXP3* gene are associated with increased risk of relapsing-remitting multiple sclerosis Romina Dastmalchi¹. *Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University*

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BACKGROUND: Although Multiple Sclerosis (MS) is an autoimmune multifactorial disease with unknown etiology, various genetic and environmental factors are known to contribute to the pathogenesis of the disease. **OBJECTIVE:** Recent studies have confirmed that the suppressive function of regulatory T cells (T (reg)) is impaired in MS patients and that the *FOXP3* gene is a crucial transcription factor in the regulation of CD4 + CD25 + FOXP3 + Treg cells. Polymorphisms in the promoter region of the *FOXP3* gene may alter the gene expression level and, therefore, contribute to the disease susceptibility. **METHODS:** The present study aimed to investigate the possible association between single nucleotide polymorphisms (SNPs) rs3761548 and rs2232365 in the *FOXP3* gene and predisposition to MS. We conducted a case-control study on 410 patients with sporadic MS and 446 healthy controls. Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **RESULTS:** Significant differences in distribution of both rs3761548 and rs2232365 A allele were found in MS patients in comparison to controls. Haplotype frequencies were also different among the studied groups. The A-A and C-G haplotype blocks showed a significant difference between case and controls. **CONCLUSION:** we have provided further evidence for the association between genetic variations and haplotypes in *FOXP3* and MS in Iranian population

R. Dastmalchi: None.

E-P09.29

Single exon deletion in the SCN2A-gene - causative for syndromal epilepsy

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Normal 0 21 false false DE X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Normale Tabelle"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0 cm 5.4 pt 0 cm 5.4 pt; mso-para-margin-top:0 cm; mso-para-margin-right:0 cm; mso-para-margin-bottom:8.0 pt; mso-para-margin-left:0 cm; line-height:107%; mso-pagination:widow-orphan; font-size:11.0 pt; font-family:"Calibri", sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-fareast-language:EN-US;} We present the second case in literature of a child with a single

exon deletion in the *SCN2A* gene on chromosome 2q24.3 detected by array CGH. The deletion is 3.5 kb in size and encompasses the complete exon 14. The 5 years old boy was presented to the genetic counseling practice because of profound epilepsy. The parents have realized regression of psychomotor and speech development since the first epileptic seizure with status in the age of 1 ½ years and above all are concerned by the stereotypical movements and behavioral problems of their son. There are a few unspecific, phenotypical abnormalities such as broad face with frontal bossing and oral hypotonia - the growth parameters lying in the normal range. The two elder brothers as well as the parents themselves are healthy. A cousin of our patient shows a different syndrome. Segregation analysis of the parents by quantitative PCR proved the deletion to be de novo. To the best of our knowledge there is only one further patient in the literature carrying a exon 14 deletion in the *SCN2A* gene (Horvath et al, 2016) due to a frameshift mutation. The 10 years old male patient had early onset global developmental delay, intermittent ataxia, autism, muscular hypotonia, epileptic encephalopathy, and cerebral/cerebellar atrophy.

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E-P09.30

Association between COMT CNR2 and MPO genes and healthy smokers or heavy smoker schizophrenia patients in Turkish population

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Aim: This study aimed to investigate whether functional variants of COMT, MPO and CNR2 genes play any role in heavy smokers (HS) or heavy smoker Schizophrenia (HSSch) patients.

Material and Methods: We included 60 (45F-Female/15M-Male) patients with HSSch, 99 (45 F/54 M) HS, and 100 (61 F/39 M) non-smoker controls (NSC). The genotyping of COMT (rs4680 and rs6269), MPO (rs233327) and CNR2 (rs2501432) genes was determined by Polymerase

Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).

Results and Discussions: The distributions of genotypes and allele frequencies were compared among the groups. The cases with HSSch had higher COMT (rs4680) Val/Val genotype than the HS and NSC groups ($p = 0.001$ and 0.034 , respectively). The cases with HS had higher Val/Met genotype than HSSch and HNC groups ($p = 0.001$, $p = 0.033$, respectively). The frequency of Val allele was higher in HSSch than the HNC groups whereas the frequency of Met allele was higher in HS than in the HSSch groups ($p = 0.047$ and 0.001 , respectively). The cases with HS had higher COMT (rs6269) LH genotype than the HSSch group ($p=0.047$). The cases with HHS had higher CNR2 TT genotype than the NSC group ($p:0.019$). The results of the study might suggest that the Val/Val genotype of COMT gene (rs4680) is associated with an increased risk for HSSch's and Val/Met genotype is associated with an increased risk for HS's. Both the LH genotype of COMT (rs6269) and the TT genotype of CNR2 are associated with an increased risk for HS's in Turkish population. ***This study was partially supported by istanbul University ONAP-BAP (47815) program.**

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E-P09.31

The influence of TPH1 and HTR1A gene polymorphisms for a person's suicide risk

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Many molecular genetics studies confirm the key role of the serotonergic system in the pathophysiology of suicidal behavior. The aim of our study is to investigate the association of the serotonergic system genes SNPs (rs6295, rs1799913) within suicide attempters. Methods. The study included 146 patients with F10($n = 72/322$) and F30–39($n = 74/322$) diagnosis hospitalized in the Psychiatry department of the Hospital of the Lithuanian University of Health Sciences after attempted suicides. Psychiatric disorders were identified according to ICD-10-AM criteria. A random sample ($n = 322$) was formed according homogeneity from Kaunas population. The DNA was extracted from blood; its concentration was measured by spectrophotometry. *HTR1A* and *TPH1* SNPs (rs6295, rs1799913) were analyzed by real-time PCR. The results were calculated statistically by

approved logistic regression and $p < 0.005$ was significant than alpha less 0.05. Results: Suicide attempters with affective disorders (F30- F39) and *HTR1A* (*rs6295*) G/G genotype and suicide attempters with mental or behavioural disorders due to alcohol use (F10) and *TPH1* (*rs1799913*) G/T genotype had a significantly higher risk for suicidal risk. The univariate logistic regression analysis to predict a suicide risk. Table 1.

Summary Our data confirmed pathogenetic link between *HTR1A* and *TPH1 SNPs* (*rs6295*, *rs1799913*) genotypes for the patients with diagnosis F30–39, F10 and a suicide factor. Financial support for the study was provided by the Research Council of Lithuania Researcher teams' projects.

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Parameter		Diagnosis	OR(95% CI)	p value
rs6295 G/G genotype vs. C/C genotype	F30–39	2.158 (1.028– 4.528)	0.042	
rs179991 G/T genotype vs. G/G genotype	F10	2.018 (1.061– 3.839)	0.032	

E-P09.32

Deletion of *TOP3B* associated with juvenile myoclonic epilepsy

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Infantile epileptic encephalopathy regroups seizures, severe cognition abnormalities and behavior impairments. These features could evolve over time and get worse especially when the encephalopathy is pharmaco-resistant. Thus, genetic studies should provide a better understanding of infantile epilepsy syndromes. Here, we investigate the genetics of juvenile myoclonic epilepsy (JME) by studying copy number variations (CNVs) in a consanguineous family with JME. As results, we have identified a heterozygous deletion of 254-kb in 22q11 region, including the *TOP3B* gene, detected among the patient, and appears to be inherited from the father. In this case report, we discuss the implication of 22q11 region in neurodevelopmental disorders and the association of *TOP3B* gene with epilepsy.

M. Daghsni: None.

E-P09.33

Exome sequencing in a large family with Tourette Syndrome/Chronic Tic Disorder

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Whole exome sequencing was performed in ten out of seventeen members of a 3-generation non consanguineous Turkish family. We identified a novel, homozygous synonymous single nucleotide variant (*rs201566317*, C > T) in the Contactin 3 (*CNTN3*) gene that segregates with Chronic Tic Disorder and Tourette Syndrome phenotype. We conclude that this single nucleotide polymorphism is a functional variant that act in cis with the presence of certain haplotypes, yet identification of these haplotypes remains elusive and further studies exploring the existence of such haplotypes should contribute to the understanding of functional significance of this low penetrance allele for the development of Tourette Syndrome. Grant Reference: The Scientific and Technological Research Council of Turkey, TUBITAK, 116S086

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E-P09.34

STC2 gene variants in Bulgarian Tuberous sclerosis cases

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Tuberous sclerosis complex (TSC) is a neurocutaneous disorder based on well-established clinical criteria. Here we report four patients, some of which with inconsistent clinical signs of TSC. NGS panel of clinically significant cancer genes was performed for all of them, targeting *TSC1* and *TSC2* genes. The first case is 4 months female with cardiac rhabdomyoma, multiple hypopigmented lesions and seizures, which coincides with the TSC clinical criteria and a nonsense variant c.4830 G > A, p.Trp1610* in the *TSC2* gene. The second case is a 43 years old man with compatible TSC clinical picture: facial angiofibromas, two hypomelanotic macules, generalized seizures and periventricular calcifications. We detected rare pathogenic variant c.2838-122G > A (*TSC2* gene). The remaining cases did not fulfil the clinical criteria, but because of the clinically ambiguous

manifestations they were also subjected to NGS. A case of 2 years old female with focal epilepsy, one hypopigmented lesion and one café au lait macule revealed single base deletion, c.4473delA, p.Val1492Cysfs*84 (TSC2 gene). The mutation was inherited from asymptomatic mother representing only few hypopigmented lesions. The last case is a 27 years old female with lung mucoepidermoid carcinoma. The cancer panel genes were tested negative, with a benign variant c.275 A > T, p.Glu92Val detected in the TSC2 gene. NGS analysis can help to genetically verify the diagnosis both in clinically clear or ambiguous cases. Sometimes incidental findings, which do not fit to the preliminary clinical manifestation can be detected, which requires team work and clinic-genetic reevaluation. The study was partially supported by Medical University Sofia, Project №8337/2016

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E-P09.35

Identification of biallelic heterozygous UNC80 mutations in a child with neurodevelopmental disorder and Cystic fibrosis from a consanguineous family in Russian Federation

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Introduction: Comorbidity of two recessive disorders in a child from consanguineous family of first cousins: cystic fibrosis (CF) (OMIM#219700) and Infantile hypotonia with psychomotor retardation and characteristic facies-2 syndrome (OMIM#616801).

Materials and Methods: The boy was born after 40 weeks of an uneventful pregnancy (3310 g/51 cm). Developmental delay and muscular hypotonia presented from birth. CF was diagnosed by the neonatal screening, confirmed by detecting of homozygous mutations in CFTR gene (p.Gly85Glu). At the age of 7 years he demonstrates muscular hypotonia, dyskinesias, distal spasticity, disuse-induced muscle atrophy. Inability to walk independently and very poor speech. Mild facial dysmorphisms: high forehead, hypotonic face, open mouth.

Results A clinical exome sequencing using Illumina TruSight One revealed two novel missense heterozygous mutations in UNC80 gene: p.Gln602His and p.Gly1262Ser, variants in highly conservative regions predicted to be pathogenic by bioinformatic tools. Sanger sequencing

confirmed this mutations in the proband and demonstrated that his parents carry those mutations in heterozygous state. Biallelic mutations in UNC80 have recently been described among individuals with an overlapping phenotype. Therefore it expected to be causative for this phenotype.

Conclusions Neurodevelopmental disorders is highly heterogeneous group, with variable clinical presentation, that makes exclusively clinical diagnostic approach inefficient. The present study emphasizes the clinical utility of exome sequencing as a first-line diagnostic test in neurodevelopmental disorders, especially in consanguineous families. This report expands the disease spectrum associated with UNC80 mutations.

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E-P10 Neuromuscular disorders

E-P10.02

Point mutations of dystrophin gene in a group of Romanian DMD patients and their female relatives

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Duchenne muscular dystrophy (DMD) is an X-linked recessive muscular dystrophy produced by degeneration of muscle fibers which determines progressive wasting of skeletal muscles. DMD is caused by mutations in dystrophin gene: complete gene deletion, deletion, duplication of

one or more exons, and point mutations. Recently, a mutation specific therapeutic approach, stop codon read through, aims to restore the expression of truncated dystrophin protein for patients with nonsense mutation. Therefore, the need for genetic diagnosis and curated database was highlighted. Our work was performed by Sanger sequencing on a group of DMD patients and their female relatives, and also on symptomatic female carriers and fetal DNA. The location (exon or intron/exon boundary), distribution along the gene and frequency (in national and international database), type (substitutions, deletions, duplications, insertions/deletions), de novo or familial, and the predicted effect on protein were assessed. The analysis revealed the increasing need for genetic and epidemiological data in order to improve access to available or future treatments and for a better outcome of medical DMD management.

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E-P10.04

The case of the combination of limb-girdle myopathy type 2 A with of connective tissue metabolism disorder, hyperhomocysteinemia

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Introduction: limb-girdle myopathy - a group of progressive muscular dystrophy, which is characterized by isolated or predominant involvement of shoulder and pelvic limb girdle muscles. For most diseases identified genes which have mutations.

Materials and methods: boy 7 years old, complained of fatigue, recurrent pain in mesogaster. Poor keeps his head, does not walk alone, contractures of large joints, limbs amyotrophy, increase of lymph nodes. In 6 years, was hospitalized with a diagnosis of functional dyspepsia. Revealed an increase of AST, ALT, CPK; bilirubin is

normal. Electromyography - reduction of the maximum activation of muscles with an emphasis on the lower leg flexors, extensors hip flexors and shoulder. The indicators of the amplitude reducing decreased to 15 - 30%. Excluded autoimmune hepatitis. In dynamics noted appearance of hypertrophy of leg muscles; a gradual increase of fatigue.

Results: Molecular analyzes: SARN3 sequenced gene (NM 000070.2) - revealed polymorphism c.550del (p. Thr184Argfs 36), c.2243GA (p.Arg748Gln) - heterozygote. Folate cycle enzymes: MTHFR 677 C/T gene - heterozygote. Biochemical tests of blood: high level of CPK, lactate dehydrogenase, elevated lactate, AST, ALT, homocysteine levels. In urine - increase the level of oxyproline.

Conclusions. In this observation myopathy syndrome combined with metabolic disorders of connective tissue, hyperhomocysteinemia. Developed a scheme of individual rehabilitation.

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E-P10.05A rare person with Stiff Person syndrome

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Stiff Person syndrome is a very rare and severe neuromuscular condition, with a prevalence estimated at 1/1000000. It is characterized by trunk and limb stiffness, painful muscle spasms, having a significant impact on the ability of performing daily activities. The exact cause is not known, now it is considered to be an autoimmune disease. Usually, the onset of the disease symptoms is in the third to fifth decade of life. We present the case of a 44 years old woman, Caucasian, hospitalized for treatment and functional rehabilitation in Medical Rehabilitation Clinical Hospital Baile Felix, Romania. At 36 years she was diagnosed with Stiff Person syndrome on the basis of clinical features, characteristic electromyogram and presence of anti-GAD antibodies. She presented in our clinic for gait difficulty, painful muscle spasms in the legs, unsystematized paresthesias, headache, dorso-lumbar pain, psychic lability. Upon admission, her physical examination revealed the following abnormal data: marked dorsal kyphosis, cervical and lumbar paraspinal muscle contracture, antalgic limited spinal mobility, abolished Achilles reflexes, exaggerated patellar tendon reflex, bilateral nystagmus, positive Romberg's test, motor control in legs type proximo-distal controlled mobility, and spastic gait with frequent imbalances. The patient also shows depressive elements. In the absence of pathogenetic treatment, pharmacologic therapies help to control symptoms. Medical rehabilitation targets to reduce spasticity, pain, improve gait and stability.

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E-P11 Multiple Malformation/anomalies syndromes

E-P11.01

16p11.2 microdeletion and microduplication in two Lithuanian patients with speech delay

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Introduction: Recurrent 16p11.2 microdeletion and microduplication syndrome is a well described condition with various expression of neurocognitive features and congenital malformations. Incomplete penetrance of the affected genes and variable expressivity usually is indicated as cause of clinical features. Here we present two unrelated patients from Lithuania with opposite CNVs in the 16p11.2 region. **Methods and results:** SNP-CGH analysis using Illumina HumanCytoSNP-12v2.1 BeadChips has been performed. 571 kb microdeletion at 16p11.2 (29628020_30199805 hg19) has been detected in the first male patient who has expressive and receptive language delay with minor facial and behavioral abnormalities. Smaller 366 kb microduplication of almost the same chromosome 16 region (29833488_30199805 hg19) has been detected in the second female patient with severely delayed speech development, global psychomotor retardation, hypernasal speech, muscular hypotonia and dysmorphic features. **Discussion:** 16p11.2 region is a variable site with many clinically insignificant small CNVs detected. Larger CNVs, encompassing disease related genes, are subject for interpretation on clinical significance when neurological features are manifested. 16p11.2 CNVs are often detected in ASD patients but our patients do not manifest any similar features. Delayed speech is the only common clinical feature in both of our patients and it is one of most frequent features indicated in patients with 16p11.2 CNVs. Involvement of this genomic region in speech development has been confirmed by several studies although candidate genes have not been determined yet. Five disease related genes are

located in affected region and only haploinsufficiency sensitive *TBX6* has indirect effect on neural development.

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E-P11.0222q11.2 distal deletion syndrome: case presentation and review of the relevant literature

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Introduction: The 22q11.2 microdeletion syndrome, the most common submicroscopic chromosomal deletion syndrome, involves a complex genomic region with eight low copy repeats(LCRs) (named A to H). Approximately 90% of 22q11.2 microdeletion cases have a common 3 Mb deletion, spanning from LCR22-A to D, resulting from LCR mediated non-allelic homologous recombination. Also, distal 22q11.2 microdeletion mediated by LCR22-D to H has recently been recognized as a clinical entity. Herein, we report an additional case with a distal deletion. We also review the relevant literature.

Case: The proband, a 25-year-old man who is the first child of a healthy non-consanguineous Japanese couple, was delivered vaginally at term weighing 1865 g. Cleft lip, microtia, and aortic valve stenosis were recognized after birth. He was able to walk at age 18 months. He showed an articulation disorder at around age 4 years, and submucosal cleft palate was recognized. His intelligence quotient score by WISC-III was 77 at 14 years.

Results: The results of G-banded chromosomal analysis and fluorescence in situ hybridization analysis for the 22q11.2 deletion were normal. Chromosomal microarray testing showed arr 22q11.21q11.22(21,798,705–22,916,612) × 1(hg19).

Conclusion: This case had a 1.1-Mb deletion flanked by LCR22-D and E, and presented growth restriction and a congenital heart disease other than conotruncal defect, consistent with previously reported cases. It is estimated that haploinsufficiency of the *MAPK1* gene, with essential function in embryonic development, encompassed by LCR22-D and E would be responsible for these features. This work was supported by Problem-Solving Oriented Training Program for Advanced Medical Personnel: NGSD Project.

M. Matsuo: None. **T. Yamamoto:** None. **K. Saito:** None.

E-P11.03**Unusual genetic finding in a patient suspected to have Alstrom syndrome**

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Introduction. Alstrom syndrome is rare disease caused by mutations of the ALMS1 gene presenting with diabetes mellitus type 2 (DM2) beginning in childhood accompanied with retinal dystrophy and blindness. Other organs such as kidney could also be affected. Hyperandrogenism can also appear. On the other hand, COHEN syndrome is caused by mutations in the COH1 gene and presents with specific facial and body features, retinal changes, severe ambliopia, obesity with glucose intolerance and leukopenia.

Material and methods. A female patient 35 years old presented with fatigue, abdominal pain and headache. She was diagnosed with diabetes mellitus type 2 since childhood. Her vision started to fade since puberty and she was virtually blind. She was obese and her menstrual cycling was irregular and amenorrhoea followed. Patient was followed up by regular testing of HbA1c, FSH, LH, estrogens, testosterone cortisol and ocular examinations. Next generation sequencing was applied for the genetic analysis.

Results. Hypertension, retinitis pigmentosa, reasonably controlled DM2(HbA1c7%) and hyperandrogenism (high testosterone levels and reversed LH/FSH index) were detected. Genetic analysis for ALMS1 gene did not detect any mutations. However, she was a compound heterozygote for two COH1 mutations, one disease causing VPS13B: c.6656 G > A (p.Cys2219Tyr) and one neutral VPS13B: c.1028 A > G (p.Gln343rg).

Conclusion. Symptoms of Alstrom and Cohen syndrome might overlap and differential diagnosis is needed. Despite the complete genetic analysis it is not clear whether the diagnosis of Cohen syndrome can be made in this patient since she carries only one pathogenic COH1 mutation.

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E-P11.04 Natural history of 19-years-old girl with monosomy 7q36.1 → qter together with trisomy 9p21.1 → pter and microduplication of SOX3 gene

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Introduction: A combination of several different approaches, including routine karyotype, FISH, and Array CGH studies are useful in identifying genes and regions responsible for phenotype changes. We present results of 19th year's observation of phenotype changes in the girl with monosomy 7q36.1 → qter and trisomy 9p21.1 → pter. In addition, the unexpected others imbalances, which could further modify phenotype, were detected by molecular studies. **Materials and Methods:** Phenotype analyzes of the girl were performed at age of 2, 5, 8, 16 and 19 years, according to quantitative method of Munchen Dysmorphology Data Base (MDDB) Array CGH was carried out using the (OGT) CytoSure™ ISCA oligoarray set (Oxford Gene Technology, Oxford, UK), and interpreted using CytoSure Software Version 4.5.3 [hg19; GRCh37, Feb2009]. **Results:** Numerous dysmorphic features, such as asymmetric face, sloping forehead, high diffuse frontal hairline, hypertelorism, flat nasal bridge, thick tip of the nose, short philtrum crowded, misaligned teeth, high palate, macrogenia, low set ears, hypertrichosis, lordosis, sacral dysostosis, dystonia and distinct developmental profile, with intellectual disability and delay of speech development consistent with Rethore s., HPE-3 and Currarino s. were found. We detected loss of 7.56 Mb at 7q36.1 → qter and gain of 28.05 Mb at 9p21.1 → pter, confirming karyotype imbalance as a result of paternal translocation with the breakpoint at PRKAG2/7q36.1 (MIM *602743) and the gain of 144 kb at Xq27.1 region that contained SOX3 gene could explain the phenotypic features observed in this girl. **Conclusions:** aCGH studies might be essential in elucidating the effect of genomic changes on the phenotype. Polish Grant - N/ST/ZB/16/001/1106 A.T. Midro: None. B.A. Kedra: None. B. Panasiuk: None. J. Popko: None. M. Kalinowska: None. M. Dębiec-Rychter: None.

E-P11.05**Association of WNT3 variations and risk of non-syndromic cleft lip and palate in Iranian infants**

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Introduction: Nonsyndromic cleft lip and/or palate (NSCL/P) is the most common orofacial birth defect, often attributed to ethnic and environmental differences. Linkage analyses and genome-wide association studies have identified several genomic susceptibility regions for NSCL/P. The *WNT* genes including *WNT3* are strong candidates for NSCL/P, since they are involved in regulating mid-face development and upper lip fusion. **Materials and Methods:** This study tested association of the *WNT3* single-nucleotide polymorphisms (SNPs), rs3809857 G/T and rs9890413 G/A, with the risk of NSCL/P in an Iranian population. The allelic and genotypic frequencies were determined in 113 unrelated Iranian subjects with NSCL/P and 220 control subjects using PCR and RFLP methods. A p-value of < 0.05 was considered statistically significant. **Results:** The rs3809857 GT genotype was significantly lower ($P = 0.039$, OR = 0.55, 95% CI = 0.30–0.97) in the NSCL/P (21.2%) than the control group (30.42%). For the rs9890413 G/A polymorphism, neither genotype nor allele frequencies were significantly different between the two case and control groups. **Conclusion:** Our results indicated that the *WNT3* rs3809857 GT genotype may have a protective effect against NSCL/P in Iranian population.

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E-P11.06

Evaluation of three patients with Bannayan-Riley-Ruvalcaba Syndrome

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Bannayan-Riley-Ruvalcaba Syndrome (BRRS) is a rare disorder caused by a mutation in the PTEN gene which is detected in 60% of patients with BRRS and located on chromosome 10q23.3. BRRS occurs both in an autosomal dominant and a sporadic manner. The characterized clinical findings of BRRS are multiple lipomas, macrocephaly, hemangioma, hamartomatous intestinal polyposis, developmental delay and speckled pigmentation on the male genitalia. The penile maculae are possibly the most marked and valuable characteristic for diagnosis of BRRS. The PTEN is a tumor suppressor gene that has a significant role in the molecular pathways of cellular proliferation, migration and apoptosis. In this report, we presented three

patients with BRRS. All of them were evaluated for clinical and radiological findings of BRRS and by molecular genetic analysis for PTEN mutation. The aim of the present report is to increase awareness of BRRS and emphasize that macrocephaly and genitale freckling are important clues for diagnosis.

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E-P11.07 Identification of novel compound heterozygous mutation in *BBS12* gene in an Iranian family with Bardet-Biedl syndrome using Targeted Next Generation Sequencing

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Introduction: Bardet-Biedl syndrome is a pleiotropic and multisystemic disorder characterized by Rod-con dystrophy, polydactyly, learning difficulties, renal abnormalities, obesity and hypogonadism. The disorder is genetically heterogeneous. Until now, around nineteen genes have been identified for Bardet-Biedl syndrome whose mutations are responsible for more than 80% of diagnosed cases. **Materials and Methods:** Recently, development of next generation sequencing strategy accelerates the mutation screening of target genes leads to save the cost and time. At present study the most common BBS genes (*BBS1-BBS13*) were screened using next generation sequencing in an Iranian family of a girl with symptoms of Bardet-Biedl syndrome. **Results:** Among 18 revealed mutations in Proposita, the two c.56 T > G and c.1156 C > T were novel. Additionally, the compound heterozygous *BBS12* mutations were confirmed in proposita following sanger sequencing of proposita and her parents. **Conclusion:** Although our data was present as a case report, however it suggests a new probable genetics mechanism maybe involved rather than conventional autosomal recessive inheritance of Bardet-Biedl syndrome.

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E-P11.08

Bohring-Opitz Syndrome - A case of a rare genetic disorder

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Introduction: The diagnostic challenge of Bohring-Opitz Syndrome, a rare genetic disorder has haunted clinicians for

ages. Case Presentation: Our proposita was born at term via caesarean-section with a birth weight of 1.95 kilograms. She was the third child to a non-consanguineous Malay couple and the pregnancy was complicated with Gestational Diabetes on diet control. She had mild laryngomalacia, gasto-esophageal reflux disease and seizures. Physical signs included microcephaly, hemangioma, low set ears, cleft palate, micrognathia, sacral pit with tuft of hair and the typical BOS posture. She also had retinopathy with left eye corneal ulcer. Her cardiovascular findings were atrial septal defect with patent ductus arteriosus. Investigations: Chromosomal analysis and consultation with a geneticist concluded that she had 46 xx-Bohring-Opitz Syndrome overlapped with C-syndrome. MRI brain showed hypoplasia of the Corpus Callosum. Flexible Naso-Pharyngolaryngoscope (FNPLS) showed tubular epiglottis with evidence of inflammation and reflux. Treatment: Goal-directed holistic care was the mainstay. The recurrent lung infections were managed with inpatient intravenous antibiotic therapy and assisted ventilation while enteral feeding was initiated via PEG tube. Conclusion: She succumbed to a Respiratory-Syncytial-Virus and Pseudomonas pneumonia complicated with sepsis at the age of 2 years and 11 months. The take home learning experience will undoubtedly be the management plan which focused on full family empowerment and involvement. This allowed excellent doctor patient/family relationship and significantly helped in the bereavement period.

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E-P11.09

A *BRAF* gene mutation in a patient with cardio-facio-cutaneous syndrome detected through whole exome sequencing in a Mexican patient with additional clinical manifestations

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Cardiofaciocutaneous syndrome is a disorder characterized by a peculiar facial appearance, heart anomalies, and intellectual disability. The heart defects are pulmonic stenosis, atrial septal defect, and hypertrophic cardiomyopathy. Sparse and friable hair, hyperkeratotic skin lesions, and a generalized ichthyosis-like condition could be also present. Most cases are sporadic although autosomal

dominant transmission has been reported. In the present study we describe a patient with a *BRAF* gene mutation (NM_004333.4:c.770 A > G, p.Gln257Arg) detected through whole exome sequencing and clinical manifestations of cardio-facio-cutaneous syndrome. Patient also presented microcephaly, bronchopulmonary dysplasia and esophageal atresia, clinical data not previously reported. In conclusion, we describe a Mexican patient with cardiofaciocutaneous syndrome and a mutation in the *BRAF* gene; however, our family has clinical data not previously observed in these patients, representing an expanded phenotype.

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E-P11.10

Concomitant 3q13.31 and 22q13.32q13.33 deletions and mosaic monosomy 22 in a patient with complex phenotype

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Introduction: Patients with chromosomal disorders are characterized by clinical variability, which is usually difficult to understand. We aimed to uncover the genetic bases of complex phenotype in a patient with r(22). Materials and Methods: r(22) was found by metaphase analysis. Microdeletions were detected by Agilent Technologies 60 K microarray and confirmed by qPCR. r(22) was confirmed and monosomy 22 was observed by FISH with CEP14/22 and probe for *TBC1D22A* gene located close to del22q13.32q13.33. The origin of mutant chromosome 22 was determined by SNP analysis. Results: We present a four-year-old girl with psychomotor development delay, hyperactivity, sleep disorder, and r(22), initially revealed by cytogenetic analysis. To specify the breakpoints aCGH was performed; del3q13.31 and del22q13.32q13.33 were found. Del3q13.31 contains the only gene - *TUSC7*, which expression is regulated together with neighboring *LSAMP*, associated with neuropsychiatric disorders in patients with 3q13.31 deletion (MIM615433). Deletion was inherited from healthy mother. 22q13.32q13.33 deletion leads to Phelan-McDermid syndrome (MIM606232). In our patient it appeared *de novo* at maternal chromosome 22. The

probands demonstrated symptoms both typical for patients with del3q13.31, del22q13.32q13.33 and never described in such individuals. FISH confirmed r(22) and found monosomy 22 in 8% of lymphocytes. Four live-born individuals with mosaic monosomy 22 were reported to date. All of them had multiple anomalies; the oldest was 3-year-old; two probands died shortly after birth. Conclusions: Complex phenotype in the patient can be explained by combination of deletions, clinical variability of del22q13.32q13.33, or mosaic r(22). This study was supported by Russian Science Foundation, grant 16-15-10231.

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E-P11.11A case of inherited t(4;10)(q26;q26.2) chromosomal translocation elucidated by multiple chromosomal and molecular analyses

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We present a boy with a plurimalformative syndrome with: prominent orbital arches, downslanting palpebral fissures, broad nose, short philtrum, micrognathia, abnormal ears, persistent ductus arteriosus, atrial septal defect. The GTG banding chromosomal analyse showed the formula: 46,XY,der(10)(10pter-10q26.2::4qter-4q26). The boy's mother presented a 46,XX,t(4;10)(q26;q26.2) formula. We applied also the MLPA with telomere probes (P-036 and P-070, MrcHolland®) and we discovered a 1,5 fold amplification of 4q35.2-4q35.1 segment (concordant with a partial trisomy 4q), a 2-2,5 fold amplification in 4q35.2 segment (concordant with a partial tetrasomy 4q in segment that comprise the *ZPF42* and *TRIML2* genes) and a 0,5 fold amplification of 10qter segment (concordant with a partial monosomy 10q). For a better characterization of chromosomal abnormality we applied an array-CGH with Sure Print G3 ISCA V2 CGH 8×60 K Array Kit (Agilent Technologies), NimbleGen MS 200 Microarray Scanner and NimbleGen MS 200 Software v1.1. The microarray-CGH confirmed 71,057 kb duplication in 4q26-q35.2 region, a 562 kb microdeletion in 10q26.3 region and a 795 kb triplication of 4q35.2 region. The first two abnormalities were pathogen, but for the last abnormally the clinical significance was unknown. For this reason we applied the array-CGH on both parents, and the mother presented the same 795 kb triplication of 4q35.2 region. In conclusion, by

using of different type of chromosomal analyses we elucidated the aetiology of chromosomal abnormality in child and we discovered a CNV without clinical significance in 4q35.2 region. This study was supported by founding of PN-II-PT-PCCA-2013-4-133 Program of UEFISCDI (National Romanian organism of research).

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E-P11.12

A novel pathogenic variant in the SMC1A gene in a patient with atypical Cornelia de Lange syndrome identified by whole exome sequencing

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Cornelia de Lange syndrome (CdLS) is a genetically heterogeneous disorder characterized by distinctive facial features, upper limb defects, growth retardation and developmental delay. An X-linked form of CdLS with milder phenotype, relative to NIPBL pathogenic variants, was caused by SMC1A mutations. We present a female 12-year-old patient with atypical Cornelia de Lange syndrome, who was initially suspected to a metabolic disease with transient hyperammonemia and severe mental retardation. She was born to nonconsanguineous healthy parents. She presented with TGA type III, hyperammonemia (transient), severe mental retardation, and generalized tonic seizures. Although she was treated with anticonvulsants, her epilepsy was uncontrolled. At the age of 12 years, though her phenotypic features of prominent forehead, upped ear (left), broad nose tip, crowded teeth, and slender fingers were noticed, she was still undiagnosed. Whole exome sequencing analysis in the patient and her parents was performed to make a genetic diagnosis. Then, a novel heterozygous variant, c.1495 C>T, in the SMC1A gene was found in the patient. The variant was *de novo* and was not found in dbSNP (build 135), 1000Genome and the ExAC database. The mutation taster program classified the variant as disease causing. The heterozygous variant in the female patient might be pathogenic and cause atypical phenotype of CdLS.

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E-P11.13A de novo FGFR3 mutation in Crouzon syndrome: a case report and review of the literature

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Crouzon syndrome (OMIM #123500), the most common craniofacial dysostosis, is associated with mutations in the FGFR2 (fibroblast growth factor receptor 2) gene and FGFR3 gene (Crouzon syndrome with acanthosis nigricans), and absence of major abnormalities of the hands and feet. The prevalence of this craniosynostosis is 1 in 60,000 live births with incomplete penetrance and phenotypic variability. We present clinical and molecular characterizations of a 11-years-old male patient with Crouzon syndrome suspicion referred to the Genetics Laboratory of the Clinical Emergency County Hospital, Tîrgu Mureş, Romania. The patient's clinical evaluation revealed turri-brachycephalic skull shape, exophthalmos, hypertelorism, hypoplastic maxilla, parrot-beaked nose, high-arched palate, ectopic eruption and proximal syndactyly of toes two and three. Genomic DNA sample was evaluated through MLPA (Multiplex Ligation-dependent Probe Amplification) and we have identified the presence of one allele of C749>G mutation in exon 7 of the FGFR3 gene. In addition, Muenke syndrome and Crouzon syndrome with acanthosis nigricans should be considered for the differential diagnosis. Since Crouzon syndrome is a rare disorder, any suspicion should be thoroughly investigated by DNA sequencing for the correct diagnosis, prognosis, and treatment of the patients with craniosynostosis in the future. In this report the novel mutation of a patient with Crouzon syndrome and review of the literature was performed.

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E-P11.14

Epilepsy and mild dysmorphic features in a girl with interstitial 8p22 microdeletion

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Introduction: In the literature, interstitial 8p22 deletion is reported in a small number of cases. In the majority of cases, the 8p22 deletion spans to the subtelomeric region and is associated with heterogeneous clinical phenotypes. We describe the 14-month old girl with 1 Mb interstitial deletion 8p22 born to non consanguineous parents in a family negative for neurological diseases, malformations

and developmental delay. From the 5th month of age she occasionally experienced brief unresponsiveness with hypotonia (focal complex epileptic seizures). At the age of 8 months she had one generalized tonic-clonic and two focal complex epileptic seizures. She represented mild dysmorphic features: down slanting eye fissures, epicanthal folds, flat nasal bridge, low-set ears and only one groove of the right hand and mild axial hypotonia. Brain MRI showed presence of mild signal hyper intensity in T1 sequence subcortically, temporally and parietally on both sides. Materials and Methods: aCGH analysis on peripheral blood using BlueGnome CytoChip Oligo 8 × 60 K array and FISH analysis on cultured lymphocytes with BAC probes (RP11) were performed. Results: Array CGH revealed de novo 1 Mb interstitial deletion on the short arm of chromosome 8p22, including gene SGCZ and microRNA 383. Deletion was confirmed by FISH. Conclusions: Cases with interstitial deletion 8p22, including gene SGCZ and MIR383, represent heterogeneous clinical phenotypes associated with neuropsychiatric disorders, mild intellectual disabilities, speech delay, slight psychomotor delay and minimal dysmorphic signs. The differences in size of the deletion and the percentage of overlapping region of the deletion play an important role in manifested phenotype.

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E-P11.15Derivative chromosome 10 in two siblings with dysmorphism: Significance of parental karyotyping

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Introduction: Balanced and unbalanced chromosomal rearrangements caused by change of parts between non-homologous chromosomes. Balanced rearrangements are not associated with dysmorphic findings but unbalanced rearrangements are associated with variety phenotypic abnormalities. Dysmorphic features occur owing to loss or gain of chromosomal material in form of partial monosomy and partial trisomy.

Material Method: We presented two siblings suffered from dysmorphic features such as microcephaly, low set malformed ears, asymmetric face, skin tag in front of both ears, hypotonia, congenital hip dislocation, growth and mental retardation, not walking, speech delay, hypospadias, micropenis. We have studied cytogenetic karyotype two siblings.

Results: They were found to carry unbalanced chromosomal translocations: 46;XY, der(10) (10pter → 10q26: 11q23 → 11qter). Parental karyotyping was performed due to the presence of derivative chromosome and phenotypic features. Maternal karyotype was 46;XX but paternal karyotype was 46,XY, t(10q26;11q23). The karyotype of the

father showed balanced translocation and the couple had a miscarriage.

Conclusions: Monosomy 10q, very rarely chromosomal abnormality, is associated with craniofacial dysmorphisms, growth retardation, development delay, congenital heart and urogenital defects in the literature. Trisomy 11q is associated with hypotonia dysmorphic facial features including a short nose, long philtrum, micrognathia, pre/postnatal growth retardation, speech delay, mental retardation, hypotonia, microcephaly, and cardiac, vertebral, limb and genital anomalies. Characterizing of balanced or unbalanced chromosome rearrangements is very important for patient monitoring and genetic counseling for future pregnancies.

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E-P11.16

Baraitser-Winter Syndrome in a boy with heterozygous missense mutation in the ACTB gene

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Baraitser-Winter cerebrofrontofacial (BWCFF) syndrome is a well-defined rare developmental disorder affecting multiple organ systems. It is characterized by the combination of congenital ptosis, high-arched eyebrows, hypertelorism, ocular colobomata and brain malformations, such as lissencephaly and pachygyria. Other typical features include postnatal short stature, microcephaly, mild to severe intellectual disability, seizures and hearing loss. BWCFF is caused by missense mutations in the cytoplasmic beta- and gamma-actin genes, namely *ACTB* and *ACTG1*. We report on a 7-year-old boy with BWCFF who initially presented with history of bilaterally hip, knee and elbow dislocations and cleft lip/palate, and craniofacial features. He had a friendly persona with mild developmental delay. Walked independently at age 3, first words were at age 3.5. The main clinical findings included mild intellectual disability, short stature, microcephaly, craniosynostosis, hypertelorism, macroblepharon, wide/puffy eyelids, high arched eye

brows, broad bifid nose, long philtrum, macrostomia, abnormal teeth, small/grooved chin, ear creases, short and webbed neck, pterygia of axilla, wide spaced nipples, umbilical hernia, broad thumb, brachydactyly, broad abnormal halluces and joint subluxation. Brain MRI imaging showed corpus callosum dysplasia, bitemporal atrophy and bifrontal gyral malformation. Sequencing of *ACTG1* and *ACTB* in the proband revealed the presence of one pathogenic heterozygous missense variant c.586 C>T, this variant leads to a p.Arg196Cys change in *ACTB*. This is the seventh patient reported with this mutation. The absence of epilepsy, hearing loss, genital and/or urinary system anomalies and presence of joint subluxation and valgus deformity of the thumb in our patient suggests significant phenotypical variability within individuals with this mutation.

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E-P11.17A case with chromosome 3 imbalance: duplication 3q and deletion 3p

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Introduction: The partial duplication of 3q and partial deletion 3p syndromes are rare, but clinically recognizable conditions. It has been shown to emerge from the meiotic recombination of the 3rd chromosome containing a pericentric inversion in one of the parents. The 3q + syndrome is confined in 3q26.3-q27 critical region and contains one or many of the conditions such as developmental delay, synophrys, long philtrum, up-slanting palpebral fissures, broad nose, hypertrichosis and congenital malformations such as heart diseases and genital hypoplasia. The 3p-phenotype is caused by deletions from 200 kb to 12.5 mb in 3pter-p25 region. Symptoms and severity of the conditions may vary due to the size of the loss and genes effected - a characteristic of contiguous gene syndromes - and can be manifested by psychomotor retardation, short stature, and craniofacial dysmorphic features.

Case Report: We here present a 3.5-years of age male with delayed development, dysmorphic facial features, strabismus and hirsutism. The patient has an operated congenital VSD. Cytogenetics revealed a structural abnormality as 46,XY,add(3)(p26). We tested the possible parental transmission and found out a maternal pericentric inversion. FISH analysis in subtelomeric regions showed a deletion at 3p and a duplication at 3q on the short arm of chromosome 3. Patient's resultant karyotype is 46,XY,rec(3)dup(3)(q?q26.1) inv(3)(p26q26.1)mat[20].ish rec(3)inv(3)(p26)(D3S4559-,D3S4560+)(q26.1)(D3S4560+).

Conclusion: The duplicated segment of the rearranged chromosome most probably contains the critically defined

region for this syndrome. Hence, we will carry out high resolution methods to determine the effected genes and try to establish a phenotype-genotype correlation.

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E-P11.18

A rare genetic syndrome with a new splice mutation detected in the EFTUD2 gene; mandibulofacial disostosis, guion-almeida type

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Introduction: Mandibulofacial Disostosis-Guion-Almeida Type (MFDGA; OMIM, # 610536) a rare autosomal dominant inherited disorder characterized by malar and mandibular hypoplasia, microcephaly, ear malformations, craniofacial malformations, typical facial appearance (pronounced metopic suture, up or down-slanted palpebral fissure, distinctive glabella, broad nasal bridge). The related gene of this disorder, elongation factor Tu GTP binding domain containing 2 (EFTUD2) is mapped in the 17q21.31 chromosomal location and consists of 28 exons. **Methods:** We examined Whole exome sequencing and the outcome was confirmed with Sanger Sequencing. At the same time karyotype analysis was performed to patient. **Results:** The case was consulted due to short stature and dysmorphic appearance. On the examination; microcephaly, down-slanted palpebral fissure, ptosis, broad nasal bridge, high palate, retrognathia, posterior rotate ears, short neck, chest deformity, bilateral brachydactyly, clinodactyly in the fifth finger, pes planus. Because there is no specific pre-diagnosis, whole exom DNA sequencing was done and an unreported c.2702 T>G (p.F901C) heterozygous splice region mutation was detected in the EFTUD2 gene. There is no mutation detected on father but study on mother still continues. **Conclusions:** It has been determined that the variant causing the amino acid change in the evolutionarily conserved region is pathogenic according to the in silico prediction tools. Our case has also been the first Turkish patient to have defined MFDM syndrome.

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E-P11.20

The effect of PAI-1 Gene Variants on Development of Thrombophilia in Patients with Klinefelter Syndrome

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Introduction: Klinefelter syndrome (KS) is a common sex chromosome related abnormality seen among men. KS negatively affects the formation of spermatogenesis and testosterone production. It is also increases the formation of thrombosis but molecular mechanism has not been well described. Plasminogen activator inhibitor-1 (PAI-1), also known as serpine1, is a protein which in humans is encoded by the gene SERPINE1. Elevated PAI-1 is a risk factor for the formation of thrombosis. PAI-1 gene polymorphisms in a promoter region 4 G/4 G, 4 G/5 G, 5 G/5 G were observed in patients with deep vein thrombosis. In this study; PAI-1 gene variants and its effects on patients with KS were examined. **Methods:** Forty-one KS patients (47,XXY) and 50 age-matched controls participated in our study. DNA was isolated from patients' peripheral blood and Real Time PCR was used to detect known SNPs in PAI-1 gene. In addition, PAI-1 plasma levels were measured by ELISA. **Results:** No significant difference was found between PAI-1 gene and KS ($p > 0.05$). However, significant difference was observed in PAI-1 plasma levels (high PAI-1 plasma level in KS / $p = 0.020$). Moreover, clinical features related to thromboembolism especially varicose veins were observed in KS patients excessively ($p = 0.04$). **Conclusion:** We think that PAI-1 gene variants may be affected by other mechanism. This study was supported by BAP 58210

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E-P11.21

A novel PIK3CA mutation in Megalencephaly - capillary malformation syndrome with extreme

brain overgrowth and 45,X mosaicism: related or coincidental phenomena?

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Megalencephaly Capillary Malformation syndrome (MCAP, OMIM 602501) is a rare condition characterized by megalencephaly, capillary malformations, somatic asymmetry, cortical brain malformations and developmental delay, caused by somatic *PIK3CA* mutations. A clinical diagnosis of MCAP made in a newborn girl was confirmed molecularly by detection of a mosaicism for a novel mutation in *PIK3CA* 11 years later. Cerebellar overgrowth with impending tonsillar herniation necessitated acute decompressive surgery at age nine months and was complicated by multiple cerebral/cerebellar infarctions post-operatively. Additional clinical features included: hydrocephalus, pronounced cerebral overgrowth with hemimegalencephaly, epilepsy, developmental delay, recurrent lingual hemangiomas, short stature and hypopituitarism with growth hormone and cortisol deficiency. G-banding revealed a 45,X cell line in 7% of blood lymphocytes. FISH-analysis showed 17,5% monosomy X in fibroblasts and 98% in resected cerebellar tissue. NGS-panel analysis in skin fibroblasts revealed a novel heterozygous mutation in *PIK3CA* (NM_006218.2) c.3129 G>A p.(Met1043Ile) in 23% of cells. The variant was absent in blood; analysis of cerebellar tissue is pending. Report of a *PIK3CA* patient harbouring a germline mutation in *PTPN11* led to speculation about a "second hit" mechanism - could some germline variants predispose for somatic *PIK3CA* mutations? Growth regulation is impaired in Turner syndrome, as in the RAS/MAPK pathway disorders. If the degree of *PIK3CA* mosaicism in cerebellar tissue correlates with the degree of 45,X mosaicism - would this be suggestive of a causal relationship?

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E-P11.22 Microphthalmia with linear skin defects syndrome -

MLS in a girl with mosaic interstitial deletion of Xp22.2-22.31

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Introduction: Microphthalmia with linear skin defects is an rare nevrolethalic X-dominant condition as a consequence of heterozygous mutation or deletion of critical HCCS gene in females and it is lethal in males. Critical region for MLS syndrome (OMIM 309801) has been defined to encompass the genes MID1, HCCS and ARGAP6. In our case, we describe a neonatus with unilateral microphthalmia and facial skin lesions with mosaic deletion of all three critical genes. Materials and methods: The girl was born with elective cesarean section because of fetal paroxysmal tachycardia. Clinical findings revealed anophthalmia of the left eye. The left eyelids were intact, the orbita was empty and the right eye was normal, without any abnormalities. She had typical linear skin defects on the left face, one on the left side of the neck, and two on the 3rd an 4th finger of the left hand. The other clinical findings and neurological exam were normal. US of the brain and EEG were normal. Molecular karyotyping using BlueGnome CytoChip Oligo 4 × 180 K array was performed. To confirm the array results, FISH using RPCI11-768H20 BAC clone on cultivated lymphocytes was used. Results: Approximately 18% mosaic, 3,3 Mb deletion, was detected by Array-CGH. Mosaicism was confirmed by FISH on interphase and metaphase cells in 29%: 46,XX,ish del(p22.2p22.31)(RPCI11-768H20-[60/205].arr[GRCh37] Xp22.31p22.2(8622553_11887361)x1 [0.18] Conclusions: Only two cases of mosaic MLS syndrome and only three cases of cryptic interstitial deletion covering HCCS gene were described. Our case support data that indicate the role of HCCS gene in variable eye and skin abnormalities.

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E-P11.24

De novo variant in *NF1* gene in a patient with suspected Noonan syndrome

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Introduction: Study of a patient with developmental delay and peculiar facies without café-au-lait spots. Clinically unaffected parents. Previous genetic tests performed with negative results: CGH-array, deletions in subtelomeric regions, Fragile X síndrome and Opitz-GBBB syndrome type I. RASopathies NGS-panel is requested by the geneticist. **Materials and Methods:** A NGS panel of RASopathies was performed. Paired-end sequencing 2 × 151pb using *TruSight One* kit (Illumina) in *MiSeq* (Illumina). Segregation study in proband's parents by PCR amplification and Sanger sequencing. **Results:** The proband is heterozygous for the Val1212Ile variant (c.3634 G > A) in the *NF1* gene. Parental studies demonstrated that the variant has de novo occurrence (however, a germinal mosaicism couldn't be excluded). The Val1212Ile missense change has not been previously reported as a disease-causing variant but replaces an evolutionarily conserved amino-acid residue; computational tools agree on the predicted pathogenic impact on the protein. Therefore this missense change is considered a pathogenic variant. A Noonan syndrome (NS) phenotype occurs in approximately 12% of individuals with *NF1* (Colley et al 1996) and other cases of missense variants in *NF1* associated with NS phenotype or *NF1*-Noonan phenotype have been previously described. The Val1212Ile variant is localized within RAS-GAP domain in neurofibromin as other documented *NF1* pathogenic variants related to NS (Chen et al 2014). **Conclusions:** A likely pathogenic variant with *de novo* occurrence is described in *NF1* gene: Val1212Ile. This case illustrates the usefulness of NGS analysis in molecular diagnosis of NS where distinguishing RASopathies from other developmental disorders with overlapping symptoms is a challenge.

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E-P11.26 Ophthalmic-acromelic syndrome in an infant

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Introduction: Ophthalmic-acromelic syndrome (MIM 206920) which is also known as Waardenburg anophthalmia syndrome is a rare autosomal recessive disorder characterised by ocular and skeletal abnormalities. Homozygous variants in *SMOC1* gene (SPARC-related modular calcium-binding protein 1 gene), mapped on chromosome 14q24

have been reported to be responsible for the syndrome. Here we report on an infant with the clinical diagnosis of ophthalmic-acromelic syndrome. **Case:** A 9-month-old female baby was referred to genetics department with bilateral anophthalmia and limb anomalies. She was born at 36 weeks of gestation with a birthweight of 1750 gr, to her consanguineous healthy parents. On physical examination, bilateral anophthalmia, cleft palate, single transverse palmar crease, bilateral partial cutaneous syndactyly between the 4th and 5th fingers, bilateral tibial bowing with dimples on her shins and bilateral partial cutaneous syndactyly between the 2nd and 3rd toes with oligodactyly were present. Developmental delay was also noted. Radiographs revealed partial bony synostoses between the basal portions of the 4th and 5th metacarpals, bilateral fibular transverse hemimelia, bowed tibiae, and four metatarsal bones on both feet. **Discussion:** Ophthalmic-acromelic syndrome was first described by Waardenburg in 1961 and since this first description, more than 35 cases have been reported. Clinical diagnosis of ophthalmic-acromelic syndrome in the present patient was made based on the presence of bilateral anophthalmia, cleft palate, syndactyly, oligodactyly and bowed tibiae. Sequence analysis of *SMOC1* gene has been started for molecular confirmation and to enable prenatal diagnosis in future pregnancies.

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E-P11.27 A Case with Pallister-Killian mosaic syndrome

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Pallister-Killian Syndrome (PKS) is a rare sporadic multiple congenital anomaly syndrome with unique clinical features. PKS is characterized by the mosaic presence of an extra 12p isochromosome. Cytogenetic analysis of cells obtained from peripheral blood usually gives normal results. The diagnosis is usually obtained by chromosome or molecular analysis in fibroblasts, buccal mucosal cells or bone marrow cells. We report a seven-day-old male baby referred to our clinic with coarse facial features, umbilical hernia, polydactyly, congenital heart disease. Our patient was a 3530-g male born by normal vaginal delivery at 37 weeks' gestation to consanguineous (first-degree cousin) parents. Pregnancy was uncomplicated except for polyhydramnios. Physical examination of the patient showed frontal bossing, frontoparietal pattern of alopecia, sparse eyebrows, depressed nasal bridge, telecanthus, short nose

with anteverted nostrils, long philtrum with thin upper lip, micrognathia, posteriorly rotated ears, thickened ear helices, prominent cheek, short neck. In addition to these dysmorphic facial features, preaxial polydactyly of the first toe, umbilical hernia, and sacral dimple was observed in the patient. Two-dimensional echocardiography showed bicuspid aortic valve, secundum atrial septal defect, tricuspid insufficiency. A chromosomal analysis in peripheral blood lymphocytes from the patient revealed a mosaic karyotype, 47, XY, + i (12) (p10) [4]/46, XY [16]. Isochromosome 12p was detected in 23% of peripheral blood lymphocytes' interphase nuclei by using fluorescence *in situ* hybridization (FISH) method. Pallister-Killian syndrome diagnosis was confirmed by these methods. Genetic counseling was given to the family. The early diagnosis of rare syndromes provides better case management and prognostic evaluation.

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E-P11.28

Compound heterozygosity for a rare and a novel mutation in LAMA1 gene associated with Poretti-Boltshauser syndrome

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Mutations in the LAMA1 gene are known to be associated with Poretti-Boltshauser syndrome, a rare autosomal recessive disorder characterised by cerebellar dysplasia, cerebellar vermis hypoplasia, cerebellar cysts in most patients, high myopia, variable retinal dystrophy, and eye movement abnormalities. We describe a child with global developmental delay, failure to thrive, facial dysmorphisms, ophthalmic and auditory abnormalities and a sacrococcygeal teratoma. An MRI brain at age 8 months revealed periventricular white matter signal abnormalities but no cerebellar cysts. Conventional karyotyping and CGH array were both reported normal. Whole exome sequencing (WES) of the proband and her parents was carried out with a coverage of $\geq 10x$ for over 95% of target bases (Illumina NextSeq). We identified two missense variants in LAMA1 in the compound heterozygous state in the proband: c.6822 G > C (p.Glu2274Asp) in exon 48, and a previously

unreported variant at c.7250 G > T (p.Gly2417Val) in exon 51. In-silico analysis predicts both variants to be disease causing with Mutation Taster scores of 0.92 and 0.99 respectively, and probably damaging predictions from PolyPhen2. The variant in exon 48 was found in the mother and the variant in exon 51 was found in the father, each in the heterozygous state. A detailed characterisation of the phenotypic features in the proband is provided and compared to published reports of Poretti-Boltshauser. This report expands the spectrum of clinical and genetic variation associated with this rare syndrome, and highlights the need for functional analysis of the reported variants.

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E-P11.29

Ring chromosome 18 in a patient with multiple anomalies

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Ring chromosome 18 in a patient with multiple anomalies A. Kalayci Yigin, G. Kaval, S. Daşdemir, M. Seven; Department of Medical Genetics, Istanbul University Cerrahpasa Medical School, İstanbul, Turkey. Ring chromosome 18 a rare genetic condition caused by having a extraordinary chromosome. Ring 18 chromosomes often occur de novo. A ring chromosome forms when due to deletion both ends of chromosome fuse with each other. During this process, some of the genetic materials may be lost and cause the clinical manifestations. Depending on the amount of chromosomal deletion, the clinical manifestations may be different. Here we report a 45 days old male child with cleft palate, parietal bossing, apathia, down slanting palpebral fissures, left ear pit, congenital deafness, congenital cardiac anomalies as VSD, ASD, mitral hypoplasia and pulmoner hypertension and minimal dilatation of bilateral lateral ventricles at cranial USG. GTG- banding chromosome analysis on peripheral blood lymphocytes showed the proband with de novo karyotype 46, XY, r(18). Array CGH may also be useful for further defined breakpoints at all imbalances. Key Words: Ring chromosome 18, multiple anomalies

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E-P11.30**A Case With Rubinstein-Taybi Syndrome: A Novel Frameshift Mutation in CREBBP Gene****M. Eser¹, A. Ayaz², G. Yesil³**

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Introduction: Rubinstein-Taybi syndrome (RSTS) is a developmental disorder characterized by a wide spectrum of multiple congenital anomalies and cognitive impairment. RSTS is primarily due to mutations in *CREBBP* (approximately 55% of cases) or *EP300* (approximately 8% of cases). Herein we reported a RTS patient with a novel mutation of the *CREBBP* gene (c.2057dupC) who presented with atypical facial appearance, feeding difficulties and recurrent respiratory infection.

Materials and Methods: DNA was extracted from EDTA-peripheral blood samples using a commercial kit according to the instructions of the manufacturer (Invitrogen, Carlsbad, CA, USA). The sequencing reactions were performed using the MiSeq Illumina sequencer (Illumina, San Diego, CA). Data analysis was performed by MiSeq Reporter. A written informed consent for the publication of the patient's pictures was given by his parents.

Results: The positive findings that overlaps with RSTS was microcephaly, beaked nose, broad great thumbs/halluces and short stature. Sequencing of *CREBBP* revealed a C duplication at c.2057 position in the *CREBBP* gene (c.2057dupC), a frameshift mutation predicted to result in premature termination at the 726th amino acid of CREB binding protein (p.Ala687SerfsTer*39).

Conclusion: With this paper, we reported a novel frameshift mutation which is lead to premature stop codon in *CREBBP* gene. As a result, c.2057dupC, reported in this paper enlarges the molecular spectrum of disease-causing *CREBBP* gene.

M. Eser: None. **A. Ayaz:** None. **G. Yesil:** None.

E-P11.31**Karyotyping in microarray era - still of benefit**

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Background: Differential diagnosis of overgrowth is wide and includes several known genetic syndromes. In many cases reaching final and accurate genetic diagnosis is not trivial; however, it is crucial for appropriate follow-up exams, particularly in disorders with increased risk for

malignancy. **Case report:** We describe a female toddler, first child to non-consanguineous parents. Prenatal abnormal findings included elevated human chorionic gonadotropin (HCG), polyhydramnios and fetal macrosomia. Non-invasive prenatal testing yielded normal results. The baby was born at 40 weeks of pregnancy with birth weight of 4125 gr (90th percentile), and diagnosed with developmental delay at the age of several months. Postnatal brain sonographic examination was normal. Several dysmorphic signs were noted during examination in our genetics clinic at the age of 11 months, including all growth parameters above 97th percentile, broad forehead, hypertelorism, depressed wide nasal bridge and axial hypotonia. Chromosomal microarray analysis (CMA) failed to detect pathogenic copy number variants. **Results:** As part of evaluation for possible diagnosis of Pallister-Killian syndrome, fibroblast karyotyping was performed. This examination yielded a balanced translocation 46XX,t(5;15);q35-q11.2. Breakage area on chromosome 5 included interruption of NSD1 gene, related to Sotos syndrome. **Conclusions:** Our case emphasizes the valuable role of karyotyping following normal CMA testing. Thus, microscopic chromosome analysis can be considered in cases in which routine diagnostic genetic evaluation fails to reach the correct diagnosis.

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E-P11.33**Mild phenotype of a large partial 13q trisomy due to t(9;13)**

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Partial trisomy 13q is a rare chromosomal abnormality with variable clinical phenotype. It usually results from adjacent segregation of a parental translocation or unequal crossing over of a parental pericentric inversion of chromosome 13. It is very rare occurs with de novo event. We report a 1-year-old girl with the mild phenotype including short neck, low set ears, long curled eyelashes, hypotelorism, high arched palate, micrognathia, long philtrum/thin upper lip, wide depressed nasal bridge, microcephaly and frontal bossing. Classical karyotyping and fluorescence in-situ hybridization studies revealed a large partial 13q trisomy due to 46,XX,der(9)t(9;13)(p24;q13). Her parents had normal karyotypes confirming de novo occurrence of the rearrangement. We planned further analyze by an array-comparative genomic hybridization (CGH). The identification of a chromosomal rearrangement is essential for genetic

counseling and for the possibility of prenatal diagnosis in such families.

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E-P11.34

Congenital heart disease revealing familial Velocardiofacial syndrome caused by 3 Mb deletion at 22q11 region

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The 22q11.2 deletion is a chromosomal malformation, present in several conditions including DiGeorge, Velocardiofacial, and Conotruncal Anomaly Face syndromes. The syndromic phenotype can include cardiovascular anomalies; palatal abnormalities; nasal voice; immune deficiency; endocrine dysfunctions; cognitive defects and intellectual disability; velopharyngeal insufficiency; and a characteristic craniofacial dysmorphism. We described a female child who was referred to Child Hospital Center with the diagnosis of heart disease but with no direct signs of Velocardiofacial syndrome. Familial evaluation led us to suspect that the mother could be a carrier for a 22q11 deletion. The proband, is a 06 months old girl. Her birth weight was 3,280 g, length was 46 cm and head circumference of 35 cm. Clinical examination showed round face, narrow forehead, epicanthic folds, small mouth., tetralogy of Fallot, and normal development. Clinical examination of her mother revealed long face, mid-face hypoplasia, narrow palpebral fissures, hypoplastic nasal alae, retrognathia, small and asymmetric ears, helix and anti-helix anomalous at left, slender hands and digits, long toes, hypernasal voice, speech delay, and learning disability. Clinical examination of her grandmother showed epicanthic folds, retrognathia, normal development, and cleft palate. Information from the family also revealed an individual who died at birth and presented heart disease. MLPA testing of patient, mother, and grandmother showed a deletion of 3 Mb extending from LCR22-A to LCR22-D. In presence of unusual findings within the spectrum of some purportedly known syndrome, familial examination could provide a definitive clue to definitive diagnosis, to prevention, orientation and genetic counseling. Financial Support: Fapeg

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E-P12 Cancer genetics

E-P12.02

Importance of detection of ALK gene rearrangement with FISH method in non-small cell lung carcinoma patients

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Lung cancer, the most common type of carcinoma, due to long-term tobacco usage is the leading cause of cancer deaths in the World. Detection of anaplastic lymphoma kinase(ALK) rearrangements in patients with non-small cell lung cancer (NSCLC)is highly recommended in clinical guidelines. As a result of inversion of ALK gene with EML4 gene, EML4-ALK complex occurs. EML4-ALK complex is continuously active which results in continuous cell proliferation. FISH is known as "gold standard" method in detection of EML4-ALK fusion gene, which mostly seen in adenocarcinoma. Success rates of treatment with tyrosine kinase inhibitors after detection of this complex is high. In our study we aimed to underly the outcomes of detection of EML4-ALK gene rearrangement in our clinics. In our study, we selected and analysed 50 cases with FISH method which are diagnosed as non-small cell lung carcinoma after pathologic evaluation between 2014 and 2016 at Kocaeli University Hospital. EML4-ALK rearrangement was detected in 8 (%16) cases. 48(%96) of all patients was smokers. %75 of positive cases have metastasis and %50 has positive familial history. All of the positive patients was started to have ALK inhibitors as a treatment after approval of positive results. ALK inhibitors are recognized as new treatment modalities for NSCLC patients and have prominent clinical efficacy. Appropriate selection for this treatment is critical in clinical practice. In our study we aimed to take attention to importance of detection of ALK rearrangement in our region.

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E-P12.03

Investigation of anticancerogenic effect on human colon adenocarcinoma cell line (HT-29) and antioxidant effect of *Salsola grandisspecies* which is endemic in Turkey

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Introduction: Plants constitute a primary source of traditional drugs which are highly effective in the treatment of many forms of cancer. **Materials and Methods:** Antioxidant content of *S. grandis* methanol extract (SGME) was determined spectrophotometrically. The phenolic acid composition of SGME was determined by LC-MS/MS analysis. The antiproliferative effect of SGME on the HT-29 was determined by MTT method. Following the treatment with SGME, the change in the expression levels of the genes and proteins involved in the apoptosis in HT-29 was determined by RT-PCR and Western blot. **Results:** It was shown that while its total phenolics content is the highest, SGME's lycopene content is the lowest. In the phenolic acid composition of the plant extract, vanillic acid is the component with the highest amount, while the component with the lowest amount is gallic acid. The IC₅₀ dose of the plant extract was calculated as 250 µg/mL for 48 h. The decrease was identified as 1.63 fold in the expression of the *BCL2* gene, compared to the control. The expression of *BAX*, *CASP3*, *CASP7*, *CASP8*, *CASP9*, *CYCS*, *FAS* and *TP53* genes increased to 1.52, 1.52, 1.76, 1.81, 1.46, 1.91, 1.18 and 1.79 fold, respectively, compared to the control. According to western blot analyses, the expression levels of proteins were consistent with the expression levels of the genes. **Conclusions:** It is believed that SGME would become a new therapeutic agent for cancer treatment and that it may stand out in the product-oriented applications in the pharmaceutical industry.

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E-P12.04

FISH identifies *MYC*, *BCL2* and *BCL6* gene rearrangement in B-cell Non-Hodgkin Lymphoma among Malaysian

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Introduction: Precision medicine is an emerging approach for disease prevention and treatment that takes into account individual variability in genes, environment, and lifestyles. While some advances in precision medicine have been made, the practice is not currently in use for most diseases. Targeted diagnostic tests such as fluorescence *in situ* hybridization (FISH) helps to determine treatment that is designed to patient's distinctive genetic profile. Patients are more likely to benefit from treatment since

FISH diagnostic tests offer clinicians a standardized and clinically validated method. Currently, information on the pattern and incidence of *C-MYC*, *BCL2* and *BCL6* gene rearrangement in B-cell Non-Hodgkin Lymphoma (NHL) are not available for Malaysian lymphomas, which preclude the application of such knowledge into the context of precision medicine. Hence, this study aims to investigate the patterns of *C-MYC*, *BCL2* and *BCL6* gene rearrangement in Malaysian B-cell NHL using FISH. **Materials and Methods:** Eighty one paraffin-embedded tumour samples between years 2011 to 2015 were collected and investigated via immunohistochemistry and interphase FISH. **Results:** There was a significant difference detected between the nodal and extranodal sites for *BCL2* ($p = 0.01^{**}$), *C-MYC* ($p = 0.03^*$) and *IgH* ($p = 0.006^{**}$) genes suggesting that there is an association between the lymphoma sites and the gene rearrangement. **Conclusions:** Undoubtedly, there is more to be learned of the B-cell pathogenesis arising at different lymphoma sites and through FISH-based tests, accurate cancer diagnostics can help determine the effective therapy for patients. This work was funded by Malaysian Ministry of Higher Education (MOHE) Fundamental Research Grant Scheme (FRGS) FRGS/1/2014/SKK06/UCSI/01/1.

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E-P12.05

Investigation of Cytotoxic and anti-proliferative effects of Betamethasone valerate (BMV) on MDA-MB 231 breast cancer cell line

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Glucocorticoids play as an essential role in embryonic development, tissue homeostasis, possess anti-inflammatory and immunosuppressive. In breast cancer, glucocorticoids may have diverse effects and could inhibit chemosensitivity. Betamethasone valerate (BMV) is a synthetic glucocorticoid that is commonly used to treat psoriasis and dermatitis. The aim of this study is to explore the cytotoxicity effects and cell proliferation of using BMV in MDA-MB-231 breast cancer cell line. MDA-MB 231 breast cancer cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing Fetal Bovine Serum (FBS) 10% (v/v) and penicillin-streptomycin 1% (v/v) and incubated at 37°C in a humidified 5% CO₂ incubator. MTT assay was used to determine the cytotoxic effect range of dosage BMV (5–150 µM). Survival assay was used for

determining the cytotoxic effect of drug for 24, 48 and 72 h. After drug treatment we observed the amount of cell viability for 65 µM BMV by 61%. We treated the cells with BMV at 24, 48, 72 h. BMV has been showed cytotoxic effects on cells after the 24 h. In this study, we investigated how glucocorticoids play a role in breast cancer and we used BMV on MDA-MB-231 breast cancer line for the first time in literature. This results indicated that BMV treatment decreased the cell viability on MDA-MB 231 breast cancer cell. In the future understanding the mechanism of BMV will be useful for cancer treatment.

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E-P12.06

Lack of BRAF V600E mutation in a Tunisian colorectal adenocarcinoma cohort

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Colorectal cancer is a heterogeneous disease with multiple underlying causative genetic mutations. The BRAF proto-oncogene plays an important role in the MAP Kinase signaling pathway during colorectal tumorigenesis. The presence of the V600E mutation at the BRAF gene influences the response of a tumor to chemotherapy. However, the association between the V600E mutation and the clinicopathological features of CRC remains controversial. Molecular genetic analysis of KRAS, and BRAF genes was carried out in a cohort of 34 Tunisian colorectal adenocarcinoma specimens. BRAF V600E mutation was investigated using extracted DNAs from formalin-fixed paraffin-embedded tumor tissues and sequencing of a short fragment of 80 pb of BRAF exon 15. Whereas, frequent mutations (in 20% of cases) with four heterozygous variants (G12D, G12S, G12V and G13D) were detected in KRAS, no mutation in the BRAF gene was detected. A recent meta-analysis revealed that the overall BRAF V600E mutation frequency is 10.8 percent in the literature and that the V600E mutation was significantly associated with advanced TNM stage, poor differentiation, mucinous histology, and tumors

localisation (proximal colon). There was a significant association between BRAFV600E mutation and wild-type KRAS. More over, many authors consider that V600E mutation could be used to supplement standard clinical and pathological staging for the better management of individual CRC patients, and could be considered as a poor prognostic marker for CRC. Further studies are justified to examine the prognostic and/or predictive value of this marker in different stages of Tunisian colorectal cancer patients.

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E-P12.07

BRCA mutation characteristics in a series of index cases of breast cancer independently of family history

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Introduction: *BRCA1* and *BRCA2* germ-line mutations were associated with high risk of breast and ovarian cancer. However, it is still uncertain how accurate estimates of cancer risk were. We, therefore, undertook to determine the prevalence of mutations in unselected ascertainment irrespective of family history.

Material and Methods: We pooled 129 index case patients with female breast cancer, unselected for family history. *BRCA1* and *BRCA2* mutation analysis were performed by Next Generation Sequencing. We analyzed all exons and exon-intron connections as well as untranslated regions of *BRCA1* and *BRCA2* genes.

Results: Among all cases, only one of the mutation carriers had a family history of breast cancer. We have identified pathogenic mutations in 18 of 129 cases (14%). The proportion of *BRCA* mutations were; 39% (n = 7) in *BRCA1* gene and 61% (n = 11) in *BRCA2* gene. Moreover, 10 of 11 *BRCA2* mutations (90%) were novel mutations that

40% (n = 4) were deleterious and 60% (n = 6) were SNVs (single nucleotide variations).

Conclusion: Mutation analysis is more often suggested for specific cases with high family risk. However, even in this small case series, our results showing the importance of general mutation screening would identify many carriers.

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E-P12.08
Deletion of exon 8 in the *BRCA1* gene in the Czech population

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Introduction: Breast cancer is the second most common cancer disease among women in the Czech Republic with incidence of 123.9/100000 women per year. Hereditary breast cancer represents approximately 10% of cases. We tested 620 Czech families suspected for hereditary breast and ovarian cancer syndrome for the presence of LGRs in *BRCA1/2* genes. In three families we identified a rare deletion of exon 8 in the *BRCA1* gene. Such a deletion was previously described in Algerian and Dutch populations and both deletions vary in the number of deleted nucleotides. Our aim was to specify the deletion of exon 8 and compare the data to previous results from other populations. **Methods:** Isolation of DNA from two independent blood samples was performed using a QiaGen kit. Analysis of long deletions and duplications of *BRCA1/2* genes was performed using MLPA kits Salsa MLPA P002 BRCA1 and Salsa MLPA P045 BRCA2/CHEK2. Long-range PCR and Sanger sequencing was used to determine exact length of the deletion. **Results:** Patient no. 1, with the *BRCA1* exon 8 deletion is heterozygous for a 3.5 kb deletion (NG_005905.2: g.115421_118954del) including a part of intron 7, whole exon 8 and a part of intron 8. Presented alteration differs from 2.6 kb and 5.7 kb deletions previously described in Algerian and Dutch populations. Further genetic testing revealed another two non-related Czech families again carrying exon 8 deletion of the *BRCA1* gene. Currently, we are testing its length and localization in order to determine whether it represents the same alteration as specified before.

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E-P12.09

Association between circulating vitamin D level and *FokI VDR* gene polymorphism with breast benign tumor

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Introduction. It's shown that circulating 25-hydroxyvitamin D (VD) deficiency is associated with breast cancer (BC). Besides, the VD level in BC patient's depends on polymorphism of genes involving in VD metabolism. However, there is no evidence for VD level role in breast benign tumor (BBT) that can increase a risk of BC formation and its genetic background still unclear. So, the aim of this study was to determine the level of VD in BBT women and to analyze the association between *FokI* polymorphism of *VDR* gene (rs2228570) with the BBT. **Materials and methods.** Genotyping performed on DNA samples extracted from venous blood from 247 unrelated women (mean age 36.14 ± 0.91) with BBT (N = 116) and control (N = 131) by real-time PCR with TaqMan probes. VD level determined by ELISA. Statistical analysis done with packet program SPSS Statistics v20. **Results.** The VD level (ng/ml) was significantly lower in BBT women comparing to a healthy donors (20.6 ± 0.71 and 29.8 ± 0.97 respectively) independently from age. Alleles and genotypes distribution for 2 T/C *VDR* gene polymorphism was under HWE ($p > 0.05$). No significant difference was determined in genotypes distribution between two studying groups ($p = 0.5921$). However, CC genotype carriers in BBT women but not in control group characterized by significantly lower VD level compared to other genotype ($p = 0.0276$). **Conclusion.** We didn't observe any association between *FokI* polymorphism of *VDR* gene and BBT risk but in patients with BBT characterized by lowering of VD level that depends on certain genotype carriers.

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E-P12.10**Evaluation of BRCA1/BRCA2 test results for Turkish breast cancer families**

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Introduction: Inherited mutations of BRCA1 and BRCA2 genes are the most common cause of hereditary breast and ovarian cancer (HBOC) in Turkish women. In this retrospective study we report the outcomes of BRCA clinical testing in Turkish individuals with personal and familial history of breast/ovarian cancer. Materials and Methods: 55 breast cancer patients and 51 at risk individuals undergoing BRCA1 and BRCA2 full sequencing in Marmara University, Medical Genetics Laboratory from 2015 to 2016 were included in this study. Clinical information, including demographic and personal/family cancer history data were obtained. Results: 67% (37/55) of breast cancer patients and 100% (51/51) of individuals at risk reported breast/ovarian cancer familial history. While mutations of BRCA1 and BRCA2 were detected in five and five breast cancer cases respectively, they were one and two in individuals at risk. So, while mutation rate in these selected breast cancer cases was 17.5% (10/57), it was 5.7% (3/52) in the risk group. Conclusion: Our results show a high frequency of mutations in our breast cancer patients. We can conclude that the criteria of the patient selection was successful. Although NGS is an efficient method for BRCA1 and BRCA2 mutation screening, many other genes need to be sequenced in HBOC cases. Besides this, increasing the number of analysed genes will increase the number of variants of uncertain significance in clinical testing.

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E-P12.11**BRCA1 and 2 sequence alterations in our patient series**

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Introduction: Pathogenic variants in BRCA1 and 2 are associated with hereditary female and male breast cancer and ovarian cancer. Less commonly, BRCA2 pathogenic variants are associated with prostate cancer, pancreatic cancer, and melanoma. **Materials and methods:** We investigated BRCA1 and BRCA2 genetic alterations. Sequence analysis and Multiplex ligation-dependent probe amplification (MLPA) methods were implemented. **Results:** We found a pathogenic variant in 3 patients (9%). Of them, c.2800 C>T and c.5194-12 G>A are known pathogenic variants, whereas BRCA1 exon 8 duplication is a novel alteration, which we presume it may cause breast cancer. Exon 8 duplication was also observed in a breast cancer diagnosed female relative. In remaining patients, non-pathogenic variants were found in heterozygous or homozygous condition. BRCA1: Q356R (7%), D693N (12%), P871L (19%), E1038G (19%), S1040N (2%), K1183R (17%). BRCA2: c.-26G>A (14%), N289H (5%), N372H (17%), R841G (2%), D1420Y (2%), T1915M (2%), T1954A (5%), K3326* (2%), *14 C>T (5%). Of these, T1954A and *14 C>T are not assigned any information in relation to breast cancer. But we observed these variants in healthy individuals of patients' relatives, a finding in favor of a benign effect. **Conclusions:** Exon 8 duplication has never been reported in a breast cancer patient before. The functional study is required to make a precise conclusion on the effect of exon 8 duplication on protein function.

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E-P12.12**The ESR1 Gene rs1801132 variation and Breast Cancer risk in Iran**

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Abstract Iranian breast cancer patients are relatively younger than their Western counterparts. Evidence suggests that alterations in estrogen signaling pathways, including *ESR1*(estrogen receptor- α), occur during breast cancer

development in Caucasians. Epidemiologic studies have revealed that age-incidence patterns of breast cancer in Asians differ from those in Caucasians. Genomic data for *ESR1* in either population is therefore of value in the clinical setting for Iranian breast cancer. A case-control study was conducted to establish a database of *ESR1* polymorphisms in Iranian women population in order to compare Western and Asian with Iranian (Asian-Caucasians) distributions and to evaluate *ESR1* polymorphism as an indicator of clinical outcome. DNA was extracted from Iranian women with breast cancer referred to Imam Khomeini Hospital Complex clinical breast cancer group (150 patients) and in healthy individuals (147 healthy control individuals). PCR single-strand conformation polymorphism technology was performed. A site of silent single nucleotide polymorphism (SNP) rs1801132 was found. The frequency of allele 1 in codon 325 (CCC → CCG) was significantly higher in breast cancer patients (39.6%) than in control individuals (28.9%; $P = 0.007$). The allele CCG had also significant association with the occurrence of lymph node metastasis. Data suggest that *ESR1* polymorphisms in exon 4 codon 325 is correlated with various aspects of breast cancer in Iran. *ESR1* genotype, as determined during presurgical evaluation, might represent a surrogate marker for predicting breast cancer lymph node metastasis. **Keywords:** breast cancer, estrogen receptor- α , SNP, PCR-SSCP. This research supported by Tehran University of Medical Sciences and Health Services grant numbers of 3054.

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E-P12.13

Comparable analysis of certain polymorphic genes relevant to methylation between the carriers and non-carriers of BRCA1 / BRCA2 mutations among the patients with familial breast cancer

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The purpose of this study was to investigate an opportunity, that mutations in genes involved in methylation may be significant risk factors for familial breast cancer. The study group of 90 women from West Ukraine diagnosed with breast and/or ovarian cancer had family history of disease. NGS analysis performed to identify pathogenic mutations in BRCA1/BRCA2 genes. PCR and RFLP analysis used to study polymorphic loci MTHFR 677 C > T, MTR 2756 A > G, TYMS 3 R/2 R, TYMS 3 R G > C, DNMT3B 149 C > T, DNMT3B 579 G > T. No significant

differences between patients and controls ($n = 90$) was found in frequency of genotypes relevant to polymorphic loci MTHFR 677 C > T, TYMS 3 R/2 R, TYMS 3 R G > C, DNMT3B 149 C > T and DNMT3B 579 G > T. The presence of MTR 2756AA genotype appeared to be associated with 2-fold increased risk of cancer (OR = 2.00, 95%CI: 1.11–3.60). The majority of carriers BRCA1/BRCA2 mutations also had MTR 2756 AA genotype (90% to 60% in non-carriers; $\chi^2 = 6.3$; OR = 6.0, 95% CI: 1.29–27.91, $p < 0.05$), whereas frequency of genotypes containing protective MTR 2756 G allele (MTR 2756 AG, MTR 2756 GG) was extremely low (10% to 40% in non-carriers; $\chi^2 = 6.3$; OR = 0.17, 95% CI: 0.03–0.77, $p < 0.05$). The results we have obtained allow to suggest that within group of patients diagnosed with familial breast/ovarian cancer, the carriers of MTR 2756 AA genotype have 6-fold increased risk bearing mutations in genes BRCA1/BRCA2, whereas presence of MTR 2756 G allele reduced this risk to 5 times. In conclusion, the carrying of MTR 2756 AA genotype seems to be highly probable risk factor for breast cancer in West-Ukrainian population.

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E-P12.14

Sphingosine-1-phosphate (S1P) receptors in non-metastatic and metastatic breast cancer patients

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Introduction: S1P receptors S1P1 and S1P3 encourage cancer progression. In this study, the aim was to evaluate the functional roles in the progression of disease by determining the expression of S1P1 and S1P3 in non-metastatic and metastatic breast cancer. Materials and Methods: Breast tissue specimens were obtained by surgery and reduction mammoplasty. The peripheral mononuclear cells (PBMC) of non-metastatic, metastatic breast cancer patients and non-cancerous individuals were also taken. Total RNA isolation

was carried out by tissues and cells. Gene expression of S1P1 and S1P3 was determined by Real-Time PCR. Results: In this study, S1P1 and S1P3 gene expressions in tissues (1.03 and 1.16 fold, respectively) were found to be higher than PBMC samples in non-metastatic breast cancer patients. In addition, in PBMC samples S1P1 levels decreased by 2.90 fold in patients with non-metastatic breast cancer, increased by 1.33 fold in patients with metastatic breast cancer. S1P3 levels decreased by 3.35 fold in patients with non-metastatic breast cancer and 1.45 fold in patients with metastatic breast cancer. Conclusions: The result of our study showed that both gene expressions were higher in cancerous tissues than in normal tissues. The expression of the S1P1 gene in PBMCs is increased in non-metastatic breast cancer patients compared to non-cancer individuals, while it is increased in metastatic breast cancer patients. S1P3 is lower in both non-metastatic and metastatic individuals than non-cancer individuals. These results indicate that, for the S1P receptors in the studies done in PBMC and tissue samples should be considered different expression properties.

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Kisspeptin Induce Aromatase Expression via Kisspeptin Receptor (GPR54) in MCF 7 Breast Cancer Cell Line

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Kisspeptin, a reproductive hormone, has been reported to have antimetastatic roles in several cancer types including colon, lung and brain, in breast cancers, however, increase in kisspeptin expression induce aggressiveness of tumors, which in turn exacerbate the breast cancer prognosis. One of the hallmarks of breast cancer prognosis is the increase in aromatase expression. The co-expression of kisspeptin and aromatase in same cells may imply an association between two. In this regard our aim was to research the possible effect of kisspeptin on aromatase expression in MCF 7 breast cancer cell line, which express both proteins. Human breast cancer cell line MCF 7 cells were treated with either 1, 10 or 100 nM of kisspeptin (Metastin) and/or Kisspeptin 2,3,4 (Kisspeptin receptor antagonist) (100 nM, 1000 nM) for 6,24 and 48 h. Aromatase (CYP19A1) and kisspeptin receptor (GPR54) mRNA expressions were determined by

Real time PCR. Actin gene was used as an internal control. Treatment with 10 nM Kisspeptin (Metastin) increased CYP19A1 and GPR54 mRNAs expressions at 6,24 and 48 h, while this inducing effect was abolished by the addition of the Kisspeptin receptor inhibitor Kisspeptin 2,3,4. In conclusion, regulatory effect of kisspeptin on aromatase expression is possibly mediated via kisspeptin receptor dependent mechanisms.

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E-P12.16

Her2 gene status in breast „in situ“ and invasive ductal carcinoma

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Introduction: The role of HER2 (*human epidermal growth factor receptor 2*) amplification/overexpression in progression of *in situ* to invasive ductal carcinoma has not been yet clarified. The aims of the study were to establish HER2 status in ductal carcinoma *in situ* associated to invasive breast ductal carcinoma. Materials and methods: Using immunohistochemical technique followed by chromogenic *in situ* hybridization were studied 100 cases diagnosed at pathology laboratory of CEDMOG, with ductal *in situ* breast carcinoma associated with invasive carcinoma. Results: Immunohistochemical staining was recorded using a semiquantitative scoring system, so 42% of cases were HER2 intense positive and 65% of cases were moderate positive. Gene amplification was found to be with high level in 17% of HER2 moderate expression cases, 42% with low level gene amplification and absence of amplification in 41% of cases. The concordance between *in situ* and invasive component of individual tumors was 88.5%. Two cases showed HER2 gene amplification in the associated ductal *in situ* carcinoma with no evidence of gene amplification in the invasive tumor. Conclusions: Multiple genetic events are required for the development of an invasive phenotype; not always HER2 gene status play a key role in the progression of ductal carcinoma *in situ* to invasive carcinoma and other molecular alterations may be more important in tumor progression. The research was made possible following completion of the project POS CCE 2.21., ID 1844, SMIS 48750, CEDMOG.

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E-P12.18

Investigation of the effect of thymoquinone on endoplasmic reticulum stress in human breast adenocarcinoma cells

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Introduction: The endoplasmic reticulum is responsible for protein synthesis and folding and has an essential role in understanding cellular stress. Recently, it has been determined that the endoplasmic reticulum mediates intracellular signaling pathways in response to the aggregation of misfolded proteins. New investigations imply that the unfolded-protein response can trigger inflammation and cancer. Thymoquinone is the active component found in the seeds of *Nigella sativa* and has been determined to have anti-inflammatory effectiveness in models of asthma, diabetes, encephalomyelitis, neurodegeneration, and carcinogenesis. Studies have revealed that thymoquinone uses its anti-neoplastic effect(s) by different modes of action. For these reasons, we aimed to investigate the effect of thymoquinone on endoplasmic reticulum stress in MCF7 and HEK293 cells. **Materials and methods:** Effective doses of thymoquinone (27 µM) in the study were determined by MTT analysis. Cells were treated with 27 µM thymoquinone for 72 h. Total RNA was isolated using TRIzol reagent. Synthesis of cDNA from the total RNA was carried out using Transcriptor High Fidelity cDNA Synthesis kit. Expression levels of genes (GRP78, CHOP, EDEM1) associated with endoplasmic reticulum stress were analyzed by RT-qPCR. Fold changes were calculated by the -ΔΔCT method. The statistical significances were analyzed applying two-tailed student's t-test and analysis of variance (ANOVA). **Results:** As a result, We found a difference in the expression levels of ER stress related genes in thymoquinone-treated MCF7 cells compared to the untreated group. This suggests that thymoquinone affects the pathways associated with ER stress.

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Combination of Long-Range PCR and Sanger sequencing leads to the identification of CHEK2 alterations in CZECH HBOC families

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CHEK2 serine threonine kinase is activated by ATM in the response to DNA damages. Simultaneously, it represents a candidate tumor suppressor gene, whose mutations leads to the molecular pathogenesis of several malignancies (prostate cancer, breast cancer, ovarian cancer, sarcomas etc.). Together with *BRCA1*, *BRCA2*, *TP53* and *ATM*, *CHEK2* plays a crucial role in the process of DNA repair, cell cycle control and apoptosis. This gene is localized at the chromosome 22q12.1, is distributed along 50 kb and comprises of 14 exons, however its analysis is technically difficult because of presence up to 16 pseudogenes. Risk of breast cancer onset in the case of *CHEK2* pathogenic mutations was estimated at 20–25% (vs. 8% in general population) and risk of prostate cancer in men at 27% (vs. 13%). Carriers of the mutations have according to published data higher risk of developing also colorectal, thyroid or renal cancer, even melanomas and osteosarcomas. According to these facts *CHEK2* gene is usually described as a low or middle-penetrant gene. Here we describe the establishment and first analysis of all coding region of *CHEK2* gene using Long-Range PCR and Sanger sequencing approach. The presentation would describe sequencing data and statistics about the presented *CHEK2* pathogenic mutations and variants of unknown significance in Czech population. We expect that the most common mutations would be presented by c.1100delC, and c.470 T>C (p. Ile157Thr) and deletion of exons 9–10 as described in other middle European studies.

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Evaluation Of Immunoglobulin Heavy Chain Region Deletions In Chronic Lymphocyte Leukemia

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B-cell chronic lymphocytic leukemia (B-CLL), also known as chronic lymphoid leukemia (CLL), is the most common type of leukemia in adults. Cytogenetic anomalies

are observed in more than 80% of cases. While many studies have been published in CLLs showing translocations involving the immunoglobulin heavy chain (IgH) region, only very few studies involving submicroscopic deletions of the IGH region have been reported. In this study, it was aimed to present the submicroscopic deletions of IGH region and their relation to clinical and treatment in patients with CLL. Patients diagnosed with CLL who applied for fluorescence in situ hybridization (FISH) analysis between January 2014 and December 2016 in Ege University Medical Genetics Department were retrospectively screened and the patients who underwent submicroscopic deletion of IGH region were included in the study. Submicroscopic deletion of the IGH region was detected in 19 patients who were diagnosed with chronic lymphocytic leukemia. The mean age of the patients was 65 and the rate of deletion was between 7% and 83% (mean: 45.34%). The clinical significance of such deletions in CLL is not fully understood. Submicroscopic deletions of the IGH gene require further studies showing clinical and therapeutic effects.

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E-P12.22 **genetic study of hereditary nonpolyposis colorectal cancer in the tunisian population**

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Lynch syndrome is an autosomal dominant disorder. It's the most common form of hereditary colorectal cancer (CRC). Genes involved belong to the mismatch repair system of DNA (MMR genes). Both of *MSH2* and *MLH1* genes present the majority of germline mutations (80%) in HNPCC. The presence of multiple genes and mutation heterogeneity are challenges for the development of diagnostic tests for this disease which is based on a set of biological, clinical, endoscopic, pathological and genetic features. In this context, we report a molecular study in Tunisian patients suspected to have the Lynch syndrome. Genetic analysis showed different variations in the concerned genes. Our study focuses on 20 patients, belonging

to Tunisian families, clinically and pathologically suspected of Lynch syndrome. In this work, we developed the optimal conditions for amplification and HRM (Hight Resolution Melting) study of all the exons of the *MLH1*, *MSH2* and *MSH6* genes, and direct sequencing exons with aberrant melting curves. We found: Three mutations in *MLH1* gene, one mutation in *MSH2* gene and no mutation in *MSH6* gene. Also, we detect fourteen polymorphisms scattered in the three genes. This work presents an initial study for nonpolyposic hereditary colorectal cancer in Tunisian patients. It will be followed by sequencing of other MMR genes (PMS2) and exome study.

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E-P12.25 **Transcriptome analysis in glioblastoma multiforme patients**

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Introduction: Glioblastoma Multiforme (GBM) is also known as astrocytoma grade IV denotes the most malignant tumors, with median survival of 6–12 months. Although etiology of GBM has not been fully elucidated, viruses like human cytomegalovirus, ionizing radiation and occupational exposure to rubber and petrochemical materials causes higher risk to that cancer. It is known that genetic factors are fundamental in development of GBMs. The alteration in the expression of the certain genes may cause of the glioblastomas. The reason why in this study, to investigate the expression of the genes in GBM patients we analysed transcriptome sequences of 10 patients. Materials and Methods: Paired normal and tumor samples were obtained from 10 patients with GBM undergoing surgery at Istanbul

University Medical Faculty Neurosurgery Department. After RNA extraction and reverse transcription, we built libraries with Ion Ampliseq Transcriptome Human Gene Expression Kit. Emulsion PCR reaction is done with Ion One Touch System and sequencing step is materialized by Ion Proton. Analysis is done by using Ion Ampliseq Plugin v5.2, Kegg Pathway Database and David Functional Bioinformatic Microarray Analysis programme. Results: Tumor and normal samples were compared and altered genes were analysed. The increased and decreased genes were determined. Conclusions: The relation between the genes and the cancer pathways are investigated. Preliminary report has been presented.

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E-P12.26

Multiple glioma in Noonan syndrome: report on a RAF1 mutated patient

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Dysregulation of the ERK/MAPK pathway is observed in one third of human cancers. Most of the genes involved in this pathway are also involved in congenital conditions called RASopathies due to heterozygote germline mutation of those genes: Noonan syndrome is caused by mutation in *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *RIT1*, *NRAS* and other rarer genes. Costello syndrome is caused by mutation in *HRAS*. Cardio-Facio-Cutaneous syndrome is caused by mutation in *BRAF*, *MAP2K1*, *MAP2K2* and *KRAS*. Neurofibromatosis type 1 is caused by mutation in *NF1*. Because of their constitutive ERK/MAPK pathway activating mutation, most of those syndromes have an increased risk of developing tumors. The estimated cumulative incidence to develop a cancer in Noonan syndrome is approximately 4%, especially during childhood. Juvenile myelomonocytic leukaemia is the most frequent but neuroblastoma, acute leukaemia and glioma are also reported. We report on a 7 year-old girl with Noonan syndrome caused by exon 12 *RAF1* mutation who developed multiple glioma located on thalamus, cerebellum and vermis. Histological examination of one of the posterior fossa tumor reveals a pilocytic astrocytomas. The other tumors have not been removed;

they have a radiological aspect of low grad glioma. We will discuss about the rare occurrence of multiple tumor in Noonan syndrome with *RAF1* mutation, the possible therapeutic approaches options and the consequences for Noonan patients follow up.

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E-P12.27

Identifying susceptibility genes associated with hereditary breast and ovarian cancer syndrome in the Faroese population

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Introduction: The aim of this ongoing study is to identify breast and/or ovarian cancer susceptibility genes - including *BRCA1* and *BRCA2* founder mutations - in the Faroese population. Faroese patients diagnosed with hereditary breast and ovarian cancer (HBOC) syndrome are routinely tested for genetic susceptibility using standard gene-panels. However, no pathogenic mutations within tested risk genes have been identified in the Faroese patients so far. In order to increase the understanding of the ethiology of HBOC in Faroese patients, we will perform whole-exome sequencing (WES) of patients and controls.

Materials and Methods: Consenting HBOC patients ($n = 50$) and control individuals ($n = 200$) will be recruited to the Faroese Genetic Biobank, and WES will be performed with a 50x coverage. Identified variants will be tested for association with HBOC. Subsequently, the top 50 variants identified from the association analysis will be tested in 150 non-cancer individuals from the HBOC families in order to determine whether the identified susceptibility variants are hereditary.

Expected results: Our goal is to identify susceptibility genes/mutations that may contribute to the cause of HBOC in the Faroese population. Further, as we are using an isolated population we may reveal rare mutations with large contribution to HBOC pathogenesis, mutations that have maintained at a lower frequency in more outbred populations.

Conclusion: The study may contribute to the development of an optimal diagnostic strategy for HBOC patients in the Faroe Islands. The project has received a grant from the Faroese Research Committee and has been approved by the Faroese-Research-Ethics-Committee.

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E-P12.28

Prevalence of HPV 16/18 amongst medical students at the University A. Neto

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Introduction: Human papillomavirus (HPV) infection is considered the most frequent transmission infection in the world. HPV is a public health problem primarily affecting young people of school age and associated with cervical cancer. **Methodology:** To analyse, the prevalence and the risk factors linked to HPV Infection 16/18 at the Medical School in Angola a cross-sectional study with cervical samples of 65 female students, mean age 23.6 that had already initiated sexual activity were obtained. The detection of the HPV genome was performed by polymerase chain reaction (PCR). **Results:** The analysis of the association between risk factors and prevalence of HPV 16/18 was significant as evaluated by Odds Ratio (OR), with 95% confidence interval and a significance level of 5%. The prevalence of cervical infection by HPV 16/18 was 26.1% (OR 2.1, IC 0654–6740), being sexually active (OR 1.1, IC 0.347 - 3.487), and with a history of STI (OR 1,438, CI 0,122–16,943). Only 1.5% were vaccinated against HPV. **Conclusions:** The high prevalence of HPV infection 16/18 and the statistically significant association with identified risk factors suggest that there is an urgent need for awareness, early screening and vaccine coverage of young women.

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E-P12.30

ERBB2 status in the in situ carcinoma associated with invasive breast carcinomas

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Introduction: The role of ERBB2 amplification/over-expression in progression of in situ from invasive ductal carcinoma has not been yet clarified. Our study evaluated ERBB2 status in ductal carcinoma in situ associated to invasive breast ductal carcinoma.

Materials and methods: 80 cases diagnosed with ductal in situ breast carcinoma associated to invasive carcinoma has been studied. ERBB2 status was determined by immunohistochemistry, followed by chromogenic or fluorescent in situ hybridization for moderate positive immunohistochemical expression.

Results: Immunohistochemically 39% of cases were ERBB2 intense positive and 60% of cases were moderate positive. The cases with ERBB2 moderate expression presented in 20% high level gene amplification, 40% low level gene amplification and absence of amplification in 40% of cases. The concordance between in situ and invasive component of individual tumors was 90%. Two cases showed ERBB2 gene amplification in the associated ductal in situ carcinoma with no evidence of gene amplification in the invasive tumor.

Conclusions: Our study emphasize that not always ERBB 2 gene amplification/ overexpression play a key role in the progression of ductal carcinoma in situ to invasive carcinoma and other molecular alterations may be more important in tumor progression.

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E-P12.31

No association between TNF alpha A308G gene polymorphism and FLT3 genes mutation in patients with acute myeloid leukemia

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Background: The A308 variant allele of tumor necrosis factor alpha (TNF α) gene was reported to influence susceptibility and outcome of patients with acute myeloid leukemia (AML). The internal tandem duplication (ITD)

and missense mutations at aspartic acid residue 835 (D835) of FLT3 gene are associated with a poor prognostic. The aim of the study was to evaluate if the TNF α A308G gene polymorphism is a risk factor for AML and if there is any correlation between the variant allele and FLT3 mutations. Material and methods: FLT3 ITD and D835 somatic mutations were analyzed in 108 AML patients while TNF α A308G polymorphism was investigated by ARMS-PCR in both patients and 150 controls. Results: In the control group we found the following genotypes: 108 GG, 40 AG and 2 AA. In the patients group 77 were GG and 31 AG. No difference was observed between the groups ($p = 0.79$). Nineteen patients were positive for FLT3 ITD, seven for FLT3 D835 from which 3 for both. Five patients were ITD or/and D835 positive and heterozygous for TNF α A308G. No association was observed between FLT3 ITD and/or D835 gene mutations and the variant allele of TNF α A308G polymorphism ($p > 0.05$). Conclusion: The TNF α A308G polymorphism is not a risk factor for AML development and is not related with FLT3 ITD and D835 gene mutations in this population. Acknowledgement: This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2016-1076 within PNCDI III, contract no 147 PED/2017.

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TERT and DNA repair gene variants as possible biological markers in Lung cancer pathogenesis and targeted treatment. A study on a Romanian Population group

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Lung cancer (LC) remains by far the most common cause of cancer-related mortality with nearly 1.6 million deaths worldwide in 2014. During the last decade, the incidence of lung adenocarcinoma has increased compared to that of squamous cell carcinoma in Romanian population as well as for other European and Asian countries. TERT plays a crucial role in cancer cell immortality and DNA repair mechanisms have a major role in genome stability therefore gene variants in these genes may represent a risk factor in lung carcinogenesis. Evidence showing correlations

between TERT retained within the nucleus and increased nuclear DNA damage sustain the fact that TERT could influence several molecules and pathways involved in inflammation, apoptosis and DNA damage responses. The purpose of our study was to investigate the possible association between *TERT*, DNA repair polymorphisms and the risk of lung cancer, in a Romanian population. For this, a group of 112 patients with lung cancer were recruited and genotyped using Real Time PCR for rs2736100 of *TERT* gene, Arg156Arg of *XPD* (ERCC2), Arg194Trp of *XRCC1* gene and Arg399Gln of *XRCC3* gene. Statistical analysis revealed that rs2736100 of *TERT* gene is associated with Arg194Trp of *XRCC1* gene and Arg399Gln of *XRCC3* gene, especially in women diagnosed with lung adenocarcinoma ($p = 0.042$). In conclusion, the results of the study suggest that polymorphisms Arg194Trp of *XRCC1* gene and Arg399Gln of *XRCC3* gene, could be associated with *TERT* mutagenesis and therefore they could represent future biological markers for the development and targeted treatment of lung cancer.

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E-P12.34

Peritoneal mesothelioma in an infant with familial ATM mutations

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Ataxia-telangiectasia (A-T) is an autosomal recessive disorder characterized by progressive neurological dysfunction, oculocutaneous telangiectasia, immunodeficiency and cancer susceptibility. The phenotype of severe A-T is caused by truncating mutations in the *ATM* gene, while mutations allowing residual protein function cause milder clinical variants. Bieloraei et al. (2013) described four A-T patients that were compound heterozygous for two rare missense mutations within exon 12 of the *ATM* gene: c.1514 T>C and c.1547 T>C, with decreased ATM expression and a predilection to T-cell Acute Lymphoblastic Leukemia (T-ALL). We now describe 6 patients of two remotely related consanguineous Bedouin families presenting with A-T. Whole exome sequencing of affected individuals identified compound heterozygosity for the two aforementioned *ATM* mutations, segregating in the affected kindred as expected for recessive heredity. While no T-ALL was evident in any of the 6 affected individuals, one

individual was diagnosed with Malignant Peritoneal Mesothelioma (MPM), an extremely rare neoplasm in pediatric patients, even in the presence of A-T. The affected individual presented with MPM at age 9 months and died at the age of 2 years. To the best of our knowledge, only a single case of MPM has been previously reported in a child with A-T, and the case reported here represents the only infant A-T patient with MPM and the youngest thus far to be described.

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E-P12.35

'Effect of ferulic acid and 5-Fluorouracil combination on expression of some metastasis genes in PC-3 cells'

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Introduction: Metastasis is associated with high mortality rate in cancer. Prostate cancer have high metastasis rate. 5-Fluorouracil (5-FU) is a pyrimidine analog and has been widely used for treatment of several cancers. The aim of the study was to determine the anti-metastatic effect of ferulic acid (FA), is abundant in fruits and vegetables, and 5-FU in PC-3 human prostate cancer cell line. **Materials and Methods:** The cytotoxic effects were determined by using XTT method after the treatment with FA, 5-FU and combination of both of them. Total RNA was isolated using TRIzol Reagent. Expressions of important genes in metastasis were evaluated by qPCR. **Results:** The IC₅₀ doses of FA and 5-FU were found to be 300 µM and 60 µM for 48 h in PC-3 cells, respectively. In subsequent analyses, PC-3 cells were treated with at IC₅₀ doses of FA and 5-FU or combination of them (200 µM FA and 40 µM 5-FU). When compared with the control group, qPCR results showed a significant decrease in the expressions of *MMP2* and *VEGFA* genes; whereas, *TIMP1* expression was increased in the FA treatment group. After the treatment with 5-FU, the expression of *CDH2* and *VEGFA* genes were significantly down-regulated. Furthermore, combination of FA and 5-FU significantly decreased expression of *MMP2*, *9*, *CDH2* and *VEGFA* genes with higher fold change compared with other groups. **Conclusions:** When compared with the single treatments of FA and 5-FU, the combination of them significantly affected expression of metastasis genes in PC-3 cells.

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E-P12.36

Effect of the nanoparticle mediated gene silencing therapy in prostate cancer: Importance of the KLK14 gene in prostate cancer development

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Kallikreins are a group of serine protease proteins, with various physiological functions. Increasing number of evidences suggest that many kallikrein subgroup proteins play a role in carcinogenesis and have the potential to be a biomarker for cancers. Early studies indicate that KLK14 expression in mRNA level differs in endocrine-induced tumours, prostate intraepithelial neoplasia and malign prostate cells. The research in nanotechnology focused on various gene therapy strategies and thus promising results are achieved for the future. These approaches include RNA interference (RNAi) and siRNA-based therapies. In this study, KLK14 gene expression was suppressed using Si RNAs in NE-1-8 cell lines, through polymeric-based nanotechnological molecules and the effects of KLK14 gene silencing on prostate cancer cells were examined. KLK14 siRNA-based gold nanoparticles were transfected into the prostate cancer NE-1-8 cell lines in three different molarities (2 nM, 4 nM, 8 nM). The level of KLK-14 gene expression was determined by performing a total RNA extraction through these cell lines. Cell survival and apoptosis rates were determined by monitoring via Xeligance system. The vitality, differentiation and apoptosis of the cells, based on potential gene silencing, were identified. Our results demonstrate that nanotechnology is possible alternative for cancer therapy.

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E-P12.37

Medullary thyroid carcinoma in a young patient with neurofibromatosis I

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Neurofibromatosis type I (NF1) is a neurocutaneous disorder caused by mutations in the NF1 gene, classified as a tumour predisposition syndrome. Due to characteristic clinical features and great size of neurofibromin gene, molecular analysis is not routinely employed. The phenotype of NF1 consist of café-au-lait spots, freckling, hamartomas, and benign and malignant neoplasms. Almost all patients present benign tumours which are neurofibromas. In about 20% of patients, intracranial malignancies appear, mainly gliomas. An increased risk of several other cancers in NF1 has been proven, including breast cancer, GIST and pheochromocytoma. About 25% of medullary thyroid carcinoma (MTC) is caused by mutation in RET gene, which may lead to MEN2A/MEN2B syndrome. We report a 20 years old patient with NF1 and MTC. The patient fully fulfils the diagnostic criteria of NF1, which include multiple café-au-lait spots and neurofibromas, plexiform neurofibromas, Lisch nodules, disseminated hamartomas of the brain, optic nerve glioma and tumours of the spinal cord. The other features are: tall stature, wide thorax, cryptorchidism and mental retardation. Because of spinal tumours, the patient suffers from tetraparesis. FISH testing for deletion in NF1 locus was performed with negative result. In the age of 17, the patient was diagnosed with MTC. Sequencing of RET gene showed no mutation. Our patient has a negative family history for NF1 and MTC. Further studies are essential to explain genetic background of this coincidence, also described in several cases in the literature.

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E-P12.38

Characterisation of the mutational spectrum of 24 Bulgarian women with ovarian cancer

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Background Ovarian cancer is a major health concern as it is one of the leading causes of cancer-related deaths amongst women. Even though pathogenic mutations predisposing to this condition are most often detected in *BRCA1* and *BRCA2*, many other genes have also been

associated with the disease. The aim of this study was to gain an insight into the mutational spectrum of 24 women diagnosed with ovarian cancer.

Materials and Methods DNA was extracted from blood and libraries were prepared for sequencing using the Illumina TruSight Cancer Panel, which targets the exons of 94 genes and 284 SNPs associated with cancer. Sequencing was performed on a MiSeq platform. Alignment and variant calling were conducted using the BWA pipeline.

Results Within our cohort of 24 women we detected pathogenic mutations in 8 patients (33%) and variants of unknown clinical significance in another 8 (33%). Six (25%) of the pathogenic mutations were identified in *BRCA1*: NP_009231.2:p.Gln1777ProfsTer74 (4 patients), NP_009231.2:p.Lys653SerfsTer47 (1) and NP_009231.2:p.Cys47Arg (1). The other two were detected in *BRCA2* - NP_000050.2:p.Val2151PhefsTer17 (4%), and in *PPM1D* - NP_003611.1:p.Leu484Ter (4%). The variants of unknown significance were identified in *BRIP1*, *DICER1*, *FLCN*, *GATA2*, *MUTYH*, *NBN* and *PALB2*.

Conclusions We detected some genetic heterogeneity within our cohort, while at the same time four women were found to carry the exact same mutation. It is important to establish the spectrum and frequency of genetic defects for a specific disease within a population, as this could pave the way for appropriate nation-wide genetic screening and targeted therapy.

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E-P12.39

Screening of the BRAF V600E mutation prevalence at papillary thyroid carcinomas (PTCs) in Turkish population

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Introduction: BRAF V600E substitution is one of the most common mutation in PTC in different populations,

and is associated with poor prognosis of the disease. The purpose of this study is to determine the prevalence of BRAF V600E mutation in PTCs in Turkish population.

Materials and Methods: Seventy-two PTC patients were enrolled in this study. DNA was extracted from FFPE tissue samples. The BRAF gene region including V600E mutation was amplified by using PCR and DNA sequencing was performed with Sanger method.

Results: A mutation at 15th exon of the BRAF gene was detected in 20 of 35 classical variant PTC patients. 16/20 of these mutations were BRAF V600E, 1/20 of these mutations was BRAF V600V and 3/20 were BRAF F583Y. At the same time two mutations were detected in 37 follicular variant of PTC patients, one being BRAF V600V, while the other BRAF V600E mutation. When mutation positive tumor samples were compared with mutation negative ones in classical variant group, thyroid capsule invasion, extra-thyroidal tissue invasion, and lymph node metastasis were associated with BRAF mutations independent from tumor size ($p < 0.05$).

Conclusions: Considering that BRAF V600E mutation is correlated with poor prognosis of the disease according to the obtained data, larger population based studies are necessary in order to follow up to prognosis of the PTC patients in Turkish population. Besides, to evaluate aggressiveness of the follicular variant PTCs and to use in distinctive diagnosis, RAS mutation screening should be considered addition to BRAF V600E in follicular variant PTC patients.

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E-P12.41 Identification of novel estrogen responsive genes differentially expressed in high-grade prostate cancer cell lines

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Estrogen receptors play an important role in cellular differentiation. The role of estrogens in prostate cancer is not fully understood. Using GEO2R and eukaryotic promoter databases as well as Dragon estrogen response (ER) element finder, among genes differentially expressed in high-grade prostate cancer cell lines, 34 novel putative ER genes were identified. Using F-MATCHTM program it was found that the promoter of the newly identified ER genes, compare to non-ER genes, were over-represented by some of the known ER elements co-exist transcription factors such as PAX3 and Sp-1. The identification of these genes

could help understanding the relationship between estrogen and prostate cancer.

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E-P12.43 RECA polymorphisms and breast cancer risk

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Breast cancer (BC) is one of the most common causes of death among women, and second in Iran. The objectives of this study were to determine the frequency of RECA G/C polymorphism in patients with breast cancer. We evaluated these polymorphisms and effects on the breast cancer risk association in a Iranian sporadic population-based case-control study of 294 breast cancer cases and 315 controls using a PCR-RFLP-based assay. Analyses of affected and controls show that homozygote genotype RECA GG has the highest frequency in both groups (33.3 in patients and 41.4 in control group). Genotype RECA GG most risk factor were in our population: [CC / GC odds ratio, 0.364 (95% confidence interval; CI, 0.168–0.788) $p = 0.009$, CC / GG odds ratio, 0.828 (95% CI, 0.411–1.668) $p = 0.596$], GG/GC odds ratio, 2.276 (95% CI, 1.497–3.460) $p = 0.001$]. There was a significant association of breast cancer risk with RECA GG and CC polymorphism.

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E-P12.45 TLR9 -1486T/C polymorphism is related to the colorectal cancer in northwestern Iran

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Introduction: The third most common cancer in the world and a leading cause of cancer death is colorectal cancer (CRC). CRC results from the progressive accumulation of genetic variations that transform normal colonic epithelium to colon adenocarcinoma. Toll-like receptors (TLRs) are a

family of transmembrane proteins that acts as innate immune sensors. TLR signaling protects the intestinal epithelial barrier and stimulates various inflammatory responses. Polymorphism located in the promoter region of TLR9 may increase colon cancer susceptibility. Materials and Methods: In total, we studied 150 patients with colon cancer and 150 healthy participants. TLR9 -1486T/C polymorphism was genotyped by RFLP-PCR. Results: There was significant difference between frequencies for TC genotype in patients and healthy individuals ($P < 0.0001$). Odds ratio (OR) and corresponding 95% confidence interval (CI) for polymorphism in TLR9 and cancer risk were estimated (OR = 3.107; 95% CI = 1.902–5.075; $P < 0.0001$). Conclusions: Our results indicate that TLR9 -1486 T/C polymorphism may be a genetic risk factor for colorectal cancer.

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E-P12.46

An investigation of polymorphisms in the IL4, IL4R and IL13 genes as risk factors for uterine leiomyomas in Slovenian women

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Introduction: The most common cause of solid pelvic tumors and for gynecological surgery in women are uterine leiomyomas (ULM). Single nucleotide polymorphisms (SNPs) might affect interleukin production and influence disease course. We investigated the associations among three SNPs in selected cytokine genes and the risk of ULM in the Slovenian population. Materials and Methods: We included 181 ULM patients, 41 women without ULM as a control group and 92 subjects representing the general population. ULM patients were divided into solitary and multiple ULM subgroups. Genotyping was performed using polymerase chain reaction followed by the restriction fragment length polymorphism technique or using high resolution melting curve analysis. Results: We found significant association between rs20541 (IL13) and ULM patients. TT genotype frequency of rs20541 was higher in healthy controls (8.7%) compared to all patients with ULM (1.0%) ($p = 0.018$). Association between SNPs and clinical characteristics of patients revealed differences between solitary and multiple ULM. In solitary ULM, rs1801275 (IL4R) was

associated with the age at first sexual intercourse ($p = 0.004$). In multiple ULM, rs1801275 (IL4R) was associated with the age at diagnosis ($p = 0.003$) where patients with the GG genotype were younger at diagnosis compared to patients with the AA or AG genotype. Conclusions: Our study suggests that rs20541 (IL13) may be associated with decreased risk for ULM development in Slovenian women. Additionally, rs1801275 (IL4R) predisposes the risk for solitary ULM in patients with a lower age at first sexual intercourse and earlier onset of disease for multiple ULM.

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E-P12.47

Role of XPC Ala499Val polymorphisms in chronic myeloid leukemia in Romanian patients

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Background: It is currently unknown whether the inherited individual capability to repair DNA damage, especially nucleotide excisions repair pathway, could affect the susceptibility to chronic myeloid leukemia (CML). The aim of our study was to evaluate the correlation between XPC Ala499Val gene polymorphism and CML risk and disease phase and EUTOS scores in a Romanian population. Material and methods: We included in this case-control study 82 CML patients and 99 healthy controls from Transylvania, Romania. Results: In the patients group the mean age was 52 years (50 men, 32 women), while in the control group 47 years (62 men, 37 women). In CML patients 40 had the wild-type genotype, 30 heterozygous and 12 variant homozygous genotype. No differences were observed regarding the genotype distribution according to the gender, ages, disease phase and EUTOS scores. In the control group the wild-type genotype was founded in 38 subjects, the heterozygous in 47 and the variant homozygous genotype in 14, with differences regarding gender distribution. In males a significant difference was observed when we compared the presence of the variant allele according to the gender between controls and patients, ($p = 0.002$, OR: 0.399, 95%CI: 0.218–0.729). Conclusion. The variant allele is not a risk factor for CML, is not related to the disease stage or outcome but in investigated population have a protective effect in males. Acknowledgement: This work was supported by a grant of the Romanian National

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E-P13 Basic mechanisms in molecular and cytogenetics

E-P13.01

Deferoxamine promotes autophagy activation and cell migration through HIF-1 α accumulation in Human Colon Cancer Cells

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Cellular adaptation to hypoxia is a protective mechanism relevant for the survival of cancer cells. Deferoxamine (DFO), an iron chelator, has demonstrated to be a potent hypoxia mimetic agent. Fe²⁺ depletion by DFO has shown to increase hypoxia-inducible factor-1 alpha (HIF-1 α) expression. HIF-1 α is a critical mediator of the physiological response to hypoxia, and its deregulation promotes tumour angiogenesis and metastasis. The present study investigated the effects of DFO in the autophagy process under hypoxic conditions in colon cancer cells. The relationship between DFO and autophagy was performed in HT29 and HCT116 cells lines. We carried out Western blot and Immunofluorescence assays to observe the expression of HIF-1 α and proteins involved in the autophagy process. Cell migration and in vitro cytotoxic effects of DFO was determined by wound healing and MTT assays. Treatment with DFO enhanced cell migration and exhibited higher expression of HIF-1 α , p62, LC3B and BECN1, associated with activated autophagy process. We also observed a decrease in the viability of the two human colon cancer cell lines. These findings suggest that DFO induces HIF-1 α and involves autophagy activation and cell migration promotion, that can play an important role in cancer cell dissemination. These results show that DFO administration in tumours with invasive potential could be an unfavourable treatment.

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E-P13.02

Rare genetic alterations involving chromosome 8: three pediatric cases

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Rare chromosomal disorders include extra, missing or rearranged chromosome material. The outcome of the affected patients can be quite different according to which chromosomes are involved. Chromosome 8 contains about 5% of the euchromatic human genome representing approx. 700 genes and its abnormalities lead to variable phenotypic features depending on the type of imbalance. In this study conventional karyotype of three unrelated patients, one male and two females, with an abnormal phenotype identified a *de novo* constitutional derivative chromosome 8, confirmed by arrayCGH and FISH techniques. We examined for them the genetic alterations of various genes in chromosome 8 to find the associations between gene copy number changes and phenotype. The patients showed structural abnormalities of chromosome 8, two cases presenting *p* arm involvement and one with unbalanced *q* arm. ArrayCGH detailed genes content of changed genetic material: a microalteration in the short arm was detected in addition to the known chromosomal imbalance for one of two patients with abnormalities of *p* arm; a region of the distal short arm containing genes involved in development or signaling of the nervous system, which presents a strikingly high mutation rate, could explain the neurologic phenotype of the third case. Results were validated using a panel of standard FISH probes. Using the latest technology smaller or complex chromosome defects can be identified. Some genomic alterations can be missed by the currently available cytogenetic techniques. Management of complex genomic imbalance involves a multidisciplinary approach and genetic counselling. *This work was supported by PN-II-PT-PCCA-2013-4-133 grant*

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E-P13.04**The associations of hOGG1 977 C > G, Lig4 26 C > T genetic variants and DNA damage in underground coal miners from Kemerovo Region (Russian Federation)**

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Introduction: coal miners are exposed to coal dust, containing inorganic and organic compounds, and to ionizing radiation. These factors can induce oxidative stress that leads to DNA damage. The amount of occupational exposure-related mutations depends on efficiency of DNA-repair protein function. Therefore it is possible to use DNA-repair genes as candidate markers for the estimation of individual susceptibility to DNA damage in coal miners exposed to hazards factors. Materials and Methods: DNA damage was evaluated using the cytokinesis-block micronucleus assay (CBMN) in peripheral blood lymphocytes. Allele-specific PCR was used to determine polymorphisms in the *hOGG1* 977 C > G (rs1052133) and the *Lig4* 26 C > T (rs1805388) genes. Exposed group included 143 coal miners (mean age = 50.11 ± 7.36 years; mean length of service in coal mining conditions = 23.26 ± 9.66 years). As a control group, we have used venous blood extracted from 127 healthy non-exposed men. Results: we discovered that coal miners are characterized by a significant increase in the frequency of binucleated lymphocytes with micronuclei (MN) ($11.15 \pm 3.81\%$), nucleoplasmic bridges (NPBs) ($4.04 \pm 2.82\%$) and protrusions (NBUDs) ($7.23 \pm 2.54\%$) compared to non-exposed donors ($7.51 \pm 1.83\%$, $2.36 \pm 1.27\%$, $5.83 \pm 2.55\%$, respectively). The T/T genotype for the *Lig4* 26 C > T and the G/G genotype for the *hOGG1* 977 C > G genes were associated with the increased frequency of binucleated lymphocytes with MN. Conclusions: we suggest that some polymorphic variants the *hOGG1* 977 C > G (rs1052133) and the *Lig4* 26 C > T (rs1805388) genes associated with the increased level of DNA damage in underground coal miners.

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E-P13.05**Recognizing 5 novel mutations in Iranian families affected by Factor VII deficiency**

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Introduction: Factor VII deficiency is a rare autosomal receive disorder involving in blood clotting in the coagulation cascade. F7 encodes a vitamin k-dependent factor which is critical in hemostasis. This disease has a phenotypic diversity from mild to severe and it was shown about one-third of individuals with factor VII deficiency never show any sign of this disease. The factor VII gene locus is on chromosome 13 (13q34). Material and methods: In the present study, mutations in factor VII gene were analyzed in a total of 26 Iranian families referred to Human genetic research center. Informed consent form was obtained and DNA extraction was done using salting out procedure. Haplotype analysis was performed in all family members using short tandem repeat (STR) markers. All exons and intron boundaries of the factor VII gene were sequenced using Sanger sequencing. Evaluating the pathogenicity of the novel mutations was done by online soft wares such as Sift, Polyphen-2, Mutation Taster, Hope Results: after observing segregation of the disease with FVII gene in the families, the gene was sequenced. It was revealed 3 novel mutations including three missense in exons 2–4, one nonsense in exon 7, and one deletion mutation in exon 8. According to the above soft wares the mutations were all pathogenic ones. Conclusion: the missense mutations might disrupt the protein structure and the nonsense and deletion caused releasing downstream part of the protein and abolished its function.

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E-P13.06**The role of ROS and gene expression in the context of mesenchymal stem cells proliferation by cerium oxide nanoparticles *in vitro***

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The ability of cerium oxide nanoparticles to stimulate stem cell proliferation *in vitro* allows considering this material as a promising cultural supplement. However, the mechanisms underlying such highly effective stimulation of proliferation remain poorly investigated. We analyzed the expression levels of a wide range of target genes in human mesenchymal stem cells with combination of cerium oxide nanoparticles by RT-PCR method. Introduction of CeO₂ nanoparticles was shown to result in an intensification of cell proliferation rate in a dose-dependent manner. The conducted analysis of the transcription profile of human mesenchymal stem cells in the presence of cerium oxide nanoparticles confirmed an increased transcription level for mRNAs of genes responsible for proliferation and cell cycle, as well as suppression of apoptosis. These data allow us to propose the assumption that cerium oxide nanoparticles can act not only as a scavenger of reactive oxygen species and to modulate the metabolic pathways functioning cells, causing the formation of a specific adaptive response. The study was funded by RFBR and Moscow City Government according to the research project № 15-34-70019 and 16-34-60248 mol_a_dk.

A. Popov: None. **N. Popova:** None. **V. Ivanov:** None.

E-P13.08

Novel Large CFTR Gene Deletions in Turkish Patients with Increased Morbidity

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CFTR is an autosomal recessive disease with a peak incidence at the populations with high consanguous marriages. Patients with only one identified mutation or with two mutations, at least one being very mild, present with classical or CF-like clinical manifestations. Large deletions of CFTR are not rare abnormalities in populations with higher consanguinity. Herein at our study we analyzed Turkish patients with large homozygote deletions at CFTR gene from the clinical point of view to compare the phenotype and genotype association. Method; We had analyzed 244 CF patients. CFTR gene is analyzed using Sanger sequencing, MLPA technique and Next generation sequencing with Illumina Miseq platform. The Exon and intron data was analyzed with Sophia Genetics version 4.2.7. Findings; the patients, 187 (76.6%) patients had at least 2 mutations whereas 31 (12.7%) patients had only one mutation detected. A total of 411 mutations and 60 variations were detected. Out of these mutations; 132 (32.1%)

were F508del mutations; 28 (6.8%) 2789 + 5 G > A, 28 (6.8%) were 2183AA- > G, 27 (6.5%) were 1677 delTA, 20 (4.9%) were N1303K. 52 (21.3%). There were 6 patients reported with large homozygote novel deletions. The age of these patients were between 12 to 23. We reviewed the phenotypic findings of the patient group and detected that the CFTR gene large deletion patient group had more oxygen support and pseudomonas infection episodes and all had pancreatic insufficiency.

P. Ata: None. **E. Atag:** None. **Y. Gokdemir:** None. **N. Bas Ikizoglu:** None. **K. Delil:** None. **E. Eralp:** None. **P. Ergenekon:** None. **B. Karadag:** None.

E-P13.09

Study the polymorphism of *lcr1* gene in *Leishmania infantum*

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Leishmania infantum is the causative agent of Mediterranean visceral leishmaniasis. There is no efficient preventive measure for this disease and its therapy is difficult with many side effects. Hence, search for a vaccine for this disease is important. *Lcr1* is an immunogenic gene discovered in *Leishmania infantum chagasi*. that causes immunologic response in human, so its molecular identification will be very important..The Aim of this thesis is to study the polymorphism of *lcr1* gene by molecular methods in MCAN/IR/2010/Meshkinshahr strain. The concluded *lcr1* sequence was compared with the reported *lcr1* sequences in other *Leishmania* parasites. *Leishmania infantum* (strain MCAN/IR/2010/Meshkinshahr) was cultured in NNN media. Parasite pellets (100 million/pellet) were prepared and genomic DNA was extracted. Parasite species was verified as *Leishmania infantum* by sequencing of ITS1 gene. *Lcr1* gene was amplified by *lcr1* specific primers. The PCR product was electrophoresed and its restriction fragment pattern was defined. Sequence of the PCR product was analyzed by Chromas and BLAST softwares and the correct sequence was determined, sequencing after gel electrophoresis. Our results show that *lcr1* gene is completely conserved in MCAN/IR/2010/Meshkinshahr strain. Conservation of this immunogenic gene (*lcr1*) adds to its value as an immunogenic molecule against

Mediterranean visceral leishmaniasis. So the importance of this identical immunologic molecule is very high.

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E-P13.10

Familial non-disjunction and segregation variation of a supernumerary marker chromosome 15 in members of a large family

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We report a familial supernumerary marker chromosomes (SMC) in a 3-months-old girl referred for craniofacial dysmorphisms (dolichocephaly, flat face, broad nasal bridge, microstomia) and normal psychomotor development. Chromosomal GTG analysis of 100 metaphases of the peripheral blood revealed a karyotype with two SMCs 48, XX, + 2mar. The father, paternal grandmother and one uncle all had the same abnormal karyotype with two copies of the same SMC. One aunt and one cousin (the son of the paternal uncle with two SMCs) had only one marker. No mosaicism of the SMC was present in any family member. SKY revealed that the SMC was derived from chromosome 15, defined as 48,XX, + 2der(15)(pterq12). The origin of SMC was confirmed by FISH. According to the literature only 11 cases showed two copies of the same marker as in this report. Derivatives of chromosome 15 represent the most common SMC and may be associated with mental and developmental retardation, seizures, autism spectrum disorder and infertility. However, our patient and all the affected family members have presented normal development, indicating that the markers might predominantly contain phenotypically silent heterochromatic domains. The acquired numerical changes in SMC inheritance in subsequent generations suggest gametic cell mosaicism in individuals with 48 chromosomes, which can be explained by a strong instability of the pairing complex during the meiotic prophase. This study has provided characterization of the unusual segregation pattern of a familial SMC, as well as identifying the origin of this marker, resulting in better clinical management for the family.

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E-P13.11

RE-EVALUATION OF A BOY WITH MONOSOMY 9pter à p22, TRISOMY 10q26 à qter

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The 9 years old male patient with known chromosomal disorder was admitted to Pediatrics Clinic because of phenotypic abnormalities. His chromosomal abnormality was diagnosed during his infancy period. His karyotype was detected 9pter -> p22, trisomy 10q26 -> qter. Both monosomy 9pter and trisomy 10q26 -> qter are rare genotypes. The patient was born at 40 weeks of gestation by vaginal delivery. Birth weight was 4190 g. He began to control his head at 6 months, sit without any support at 9 months, began walking at 25 months. He had mild dysmorphic features, such as frontal bossing, low-set ears, hypertelorism, broad flat nose, high-arched palate, short neck. A re-evaluation was conducted at the age of 9 years. His head was trigonocephalic with frontal bossing. The most common clinical features of monosomy 9p syndrome, included developmental and psychomotor delay, trigonocephaly, flat midface, short palpebral fissures, highly arched eyebrows, low-set ears, short flat nose with anteverted nostrils, thin upper lip, long philtrum, high palate, micrognathia, short neck, enlarged internipple distance, tapering fingers, flat feet, and hypotonia. Trisomy 10q is also rare syndrome. Characteristic craniofacial findings include a flat round face with full cheeks and large prominent forehead, highly arched eyebrows, short and narrow palpebral fissures (blepharophimosis), widely spaced eyes with telecanthus, a short nose, a bow-shaped mouth with a prominent upper lip, and a small mandible. Our patient has both phenotypic findings from monosomy 9q and trisomy 10q syndromes. This case is the first in the literature having these rare syndromes together.

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E-P13.12**A new case of mosaic tetrasomy 15q25.3-qter due to a supernumerary marker chromosome**

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Introduction: Supernumerary marker chromosomes (SMC) arise in approximately 0.05% neonates. Tetrasomy for the distal part of chromosome 15q (15q24-qter) due to a SMC is rare, indeed, only 23 cases have been reported in the literature so far, including 7 showing non-mosaic tetrasomy. Some SMC are reported as analphoid, they are structurally rearranged chromosomes which acquired neocentromeres allowing them to remain stable during mitoses. Although some common clinical features have been described, no clear link between tetrasomy 15q24-qter length, degree of mosaicism and patient's phenotype severity has been established. **Material and methods:** Here, we report a case of tetrasomy 15q25.3-qter identified using array-CGH, FISH and standard karyotype. The patient, a 16-year-old girl, presents intellectual deficiency, facial dysmorphism, marfanoid habitus, thin fingers, brachymetatarsy, severe scoliosis, and mitral valve prolapse. **Results:** The array-CGH analysis revealed a gain of 16.7 Mb of the distal region of the long arm of chromosome 15 (15q25.3q26.3). Surprisingly the karyotype showed mosaicism for 15q SMC: mos 46,XX[7]/47,XX, + i(15) (qter->q25.3-?:q25.3->pter)[13], confirmed by FISH: ish + i(15q)(D15S936 + +)[13]/15q26.3(D15S936x2)[12].nuc ish(D15S936x4)[14]/(D15S936x2)[11]). The FISH analysis, using a 15q26.3 region specific probe, showed one signal on each normal chromosomes 15 and two additional signals on each arm of the marker in 14/25 nuclei and 13/25 mitoses respectively. This corresponds to an inverted duplication of the distal region of chromosome 15 q arm. **Conclusion:** This newly identified case reveals a novel mosaic tetrasomy 15q and provides new data regarding this rare chromosomal abnormality and its associated clinical features.

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E-P13.13**Whole-exome sequencing in a three generation family with autosomal-dominant inherited omphalocele**

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Introduction Omphalocele is a congenital abdominal wall defect that permits herniation of abdominal viscera covered by a sac consisting of peritoneum, Whartons jelly, and amnion. The usual contents are intestine, liver, and spleen. The birth prevalence of nonsyndromic omphalocele is estimated to be 1 in 4.000 live births. Almost all cases with nonsyndromic omphalocele occur sporadic with no single genetic cause known to date. Here we performed whole exome sequencing (WES) in a three generation family with autosomal-dominant inherited omphalocele and 4 affected individuals.

Materials and Methods WES was performed on two affected and one unaffected family member. Mutation analysis was performed by WES (enrichment kit: Nimble Gene SeqCap ES Human Exome Library 2.0) with the Genome Analyzer II (Illumina). Read alignment and detection of variants was done with Genome Analyzing software (varbank, www.varbank.ccg.uni-koeln.de/).

Results Application of standard filter criteria, confirmation of variants with Sanger sequencing and segregation analysis within the family revealed two variants in *SLC46A2* (c.761 G > A) and *ALB1* (c.253 + 3 A > C).

Conclusions Functional studies are warranted to further prioritize the possible disease causing variant. **Grant reference** Caroline Maria Katharina Kolvenbach is supported by the BONFOR program of the University of Bonn (grant number O-149.0120.1).

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E-P13.15 A Complete Workflow for Human Cell Line Authentication

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The study of human development and diseases relies heavily on the analysis of dissociated human cell lines grown in culture. However, an increasingly acknowledged problem is that cells grown *in vitro* can become misidentified or contaminated with other unrelated cell lines. Analysis of highly variable short tandem repeats (STRs), provides a simple, inexpensive and highly specific genetic “fingerprint” of a cell line. In this study, we describe a complete workflow for human Cell Line Authentication by combining Thermo Fisher Scientific AmpFISTR Identifiler STR analysis kits, Applied Biosystems’ gold-standard CE instrumentation and Gene Mapper analysis software. Two different workflows were analyzed. First, serial dilutions of purified genomic DNA were isolated from a number of human cell lines were analyzed using the AmpFISTR Identifiler Plus kit. Correct allelic calls were made when the input DNA amount was between 0.3 and 3 ng. Second, dilutions of suspensions of the same cells were immobilized onto Copan Nuclie Cards, and 2 mm punches were analyzed using the AmpFISTR Identifiler Direct kit. Correct allelic calls were made when a suspension of $5-10 \times 10^5$ cells were immobilized. To test the limit of detection of contaminating cells in a culture, we prepared mixed cell suspensions of 5×10^5 cells of M4A4GFP cells and HeLa cells. Contaminating HeLa cells can be detected in a population if they are present at greater than about 25% of the total population. Together, these results describe a facile and consolidated workflow for human cell line authentication.

S. Jackson: A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **K. Varma:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific

E-P13.16 Turner syndrome's mosaicism revisited: a FISH/MCB study of 96 cases

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Sharonin², I. V. Soloviev², T. Liehr⁴, I. Y. Iourov^{2,1}; ¹Academician Yu.E. Veltishchev Research Clinical Institute of Pediatrics, N.I. Pirogov Russian National Research Medical University, Ministry of Health of the Russian Federation, Moscow, Russian Federation, ²Mental Health Research Center, Moscow, Russian Federation, ³Moscow State University of Psychology and Education, Moscow, Russian Federation, ⁴Institute of human genetics, Jena, Germany.

Introduction Turner syndrome (TS) is a well-known chromosomal disorder affecting 1/2000–1/2500 newborns. This condition is associated with a variety of karyotypes, each type of which is featured by monosomy of chromosome X. In addition, chromosomal mosaicism is likely to be responsible for 30–56% of TS cases associated with monosomy X. Materials and methods Ninety six TS (TS-like phenotype) girls were analyzed using banding karyotyping (20 metaphase plates per case or 30 metaphase plates if mosaicism revealed), FISH (50–100 metaphase plates and 1000 interphase nuclei) and MCB. Cut-off mosaicism rates (proportion of cells without chromosome X) were 2.5%. Results Regular (classic 45,X) variant was found in 28.1%. Most cases (37.5%) exhibited chromosomal mosaicism affecting the whole chromosome (45,X/46,XX(45,X/47,XXX/46,XX); ring chromosome 46,X,r(X)/45,X in 8.3%; isochromosome X 46,X,i(Xq)/45,X and marker chromosome 46,X,mar(derX)/45,X accounted for 6.25%. Less frequently TS phenotype was shown to be associated with 46,X,i(Xq), 46,X,i(Xq)/45,X/46,XX, 46,X,idic(X)(q22.2)/45,X, 46,X,mar der(Y)/45,X, 46,X,del(Yq), 46,X,der(X)t(Xp;Yq), 45,X/45,X,der(20)t(20p;Yq), 46,X,del(Xp), 46,X,del(Xp)/45,X. Conclusions The present study showed the proportion of mosaic karyotypes to be as high as 67.7%. Mosaicism was detected in 39.6% of cases. According to these data, we may conclude that mosaicism is more common in TS than previously recognized. Thus, different karyotypes observed in TS allow speculations that individual approach in each case is required to improve and individualize molecular diagnosis. Supported by the Russian Science Foundation (project #14-15-00411).

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E-P13.17**Molecular genetic basis of urticaria development**

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Urticaria is a common skin condition with pruritic raised, well-circumscribed areas of erythema and edema involving the dermis and epidermis. It affects about 20% of people at some time during their lives. Urticaria is a complex disease with pathogenesis involving allergic inflammation, skin lesions and hyperreactivity to environmental triggers. Therefore we analyzed common changes in genes of filaggrin (c.2282del4, p.R501X), cytokines (rs2243250 of IL4, rs20541 of IL13, rs1800872 of IL10, rs1800629 of TNFA) and toll-like receptors (rs5743571 of TLR1, rs5743794 of TLR6, rs11466617 of TLR10) in urticaria patients and healthy individuals from Volga-Ural region of Russia. The patients group include 103 individuals of different ethnic origin (Russians, Tatars, Bashkirs and individuals of mixed origin). The control group consists of 106 healthy donor without atopic diseases of respective ethnic background. Genotyping was performed by PCR-RFLP. We revealed that rs20541*Arg/Gln genotype of the IL13 gene ($p = 0.02$) and rs5743794*CC genotype of TLR6 ($p = 0.0011$) is associated with urticaria in Russians and TLR1 gene rs5743571*TT genotype - with urticaria in Tatars ($p = 0.0054$). Besides, rs5743794*C allele is associated with acute and chronic urticaria ($p = 0.0209$ and $p = 0.0063$, respectively) and rs2243250*C allele of IL4 gene polymorphism - with acute urticaria ($p = 0.0247$). Allelic frequency of the FLG gene c.2282del4 mutation is 0.96% in patients, 2.12% in controls, frequency of p.R501X mutation - 1.4% and 0.53%, respectively, but these differences are not statistically significant. Thus, we found an association of urticaria with rs2243250 of the IL4, rs20541 of the IL13, rs5743571 of the TLR1 and rs5743794 of the TLR6 gene.

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E-P14 New diagnostic approaches, technical aspects & quality control**E-P14.01****Challenges and solutions for FFPE DNA quantitation**

K. Plasman, T. Duthoit, T. Martens, T. Montoye

Trinean NV, Gentbrugge, Belgium

As large collections of FFPE samples have been gathered over many years, FFPE tissue derived DNA or RNA are popular subjects in genetic studies and cancer research. The success of downstream tests with DNA isolated from FFPE tissue blocks however cannot be guaranteed as samples can be fragmented, chemically altered and/or heavily contaminated with carry-over molecules from the source material and extraction buffer constituents. Proper quantification and purity analysis after isolation are therefore desirable. In this poster we present experimental data [TM1] to demonstrate the advantages and shortcomings of the most used FFPE DNA quantification technologies, being A260 absorbance, Qubit fluorescence and DropSense/cDrop. Finally, we propose ways to standardize FFPE DNA QC in order to achieve higher success-rates of downstream assays.

K. Plasman: None. **T. Duthoit:** None. **T. Martens:** None. **T. Montoye:** None.

E-P14.02**Evolving challenges of molecular diagnostics in a regional center**

M. Witsch-Baumgartner, E. Maurer, J. Zschocke

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Molecular diagnostics has shifted from institutes with special expertise for individual indications to a unified diagnostic pipeline in a regional institute. Now, each center has the possibility to perform analysis for all diseases and genes known and doing it at the regional level. This implies different initial preconditions regarding referring reasons with phenotype description, consent of patient, analysis pipeline, interpretation of results, and counseling of patients. We present a well-developed approach to consider all these circumstances and we show examples. Our approach regards the incoming sample from the referring physician asking for elucidation of a case with an initial clinical suspicion, e.g. amelogenesis imperfecta, or a patient with intellectual disability and epilepsy. Regarding molecular analysis patients should be seen in the genetic clinic or by regional partners to adequately document the phenotype and obtain consent. The method is straight forward: library preparation for massive parallel sequencing for a targeted gene panel or a clinical exome (OMIM disease genes) both

including CNV analysis depending on suspected disease or genes. Only target genes are uncovered from the panel, no irrelevant genes are analyzed to minimize challenge of interpretation and preclude incidental findings. Massive parallel sequencing is complemented by all relevant diagnostic methods including routine transcript sequencing, fragment analyses, Southern blot, DNA array, FISH. This mode of handling in our laboratory regarding diagnostics of inherited diseases permits to deliver high quality reports and answers specific questions of referring physicians; examples of successful application of the pipeline will be presented.

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E-P15 Personalized/Predictive Medicine and Pharmacogenomics

E-P15.01

Study the expression level of Bcl-2 gene in *Aloe vera* treated AGS cell line

Z. Deilami Khiabani, N. Tariverdi

Islamic Azad University, Zanjan, Iran, Islamic Republic of
 Normal 0 false false EN-US X-NONE FA /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:**; mso-padding-alt:0 cm 5.4 pt 0 cm 5.4 pt; mso-para-margin-top:0 cm; mso-para-margin-right:0 cm; mso-para-margin-bottom:10.0 pt; mso-para-margin-left:0 cm; line-height:115%; mso-pagination:widow-orphan; font-size:11.0 pt; font-family:"Calibri","sans-serif"; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:Arial; mso-bidi-theme-font:minor-bidi;} Use of anticancer herbal drugs because of having fewer side effects is of importance. *Aloe Vera* is one of herbal medicines which contain a variety of valuable minerals, vitamins, amino acids and antioxidant. In most cases these anticancer herbal medicines change the expression of variable genes. The aim of this study has been to investigating changes in gene expression of Bcl-2 in *Aloe Vera* treated human AGS adenocarcinoma cells lines. AGS cells were treated with the aqueous extract of *Aloe Vera* in different concentrations including 800,1200 and 2000 µg/ml. After 48 h and 72 h treatment, RNA extraction has been done. Following cDNA synthesis, the rate of expression of BCL-2 and BAX genes were evaluated using Real time PCR. Significant changes on Bcl2 gene expression have been shown just in 800 µg/

ml dose of *Aloe Vera* extract after 48 h treatment. Reduction of Bcl-2 gene expression could promote the gastric cancer cells to apoptosis.

Z. Deilami Khiabani: None. **N. Tariverdi:** None.

E-P15.02

A clinical approach to cardiovascular and neurodegenerative diseases: the use of family history and molecular tests in diagnosis, risk-assessment and management

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Introduction: Cardiovascular diseases (CVDs) and neurodegenerative diseases (NDDs) are among leading causes of morbidity and mortality throughout the world. There have been several reports in the scientific literature which suggest the linkage between CVDs and NDDs.

Materials and Methods: Blood samples were collected from 144 unrelated patients aged 35 - 60 years with various clinical records. Each of them had a family history of CVDs, including stroke, coronary artery disease, heart failure and etc. PCR-RFLP was undertaken to examine polymorphisms of 10 genes, associated with CVDs: APOE, NOS3, MTHFR, MTRR, ACE, PAI1IL-4, IL-4RA, TNF and CCR5.

Results: A significant correlation between the simultaneous presence of several gene polymorphisms: NOS3 (G894T or T1468A); MTHFR (C677T); APOE (ϵ 3) and the existing family history of acute ischemic stroke at early age was observed in 46 patients. However, the majority of these patients (n = 37/46; 80%) show no clinical signs of CVDs (normal LDL-C levels, blood pressure and etc.), while 30 patients (n = 30/46; 65%) present neurological disorders in their anamnesis, such as personality changes, loss of behavioral skills, minimal cognitive impairments and etc. It is remarkable that none of the mentioned patients have NDDs in their family histories.

Conclusions: It is known that variations in NOS3 gene are associated with high susceptibility to coronary spasm and MTHFR (C677T) and APOE (ϵ 3) are linked to high blood pressure. However, our research confirms that combination of these polymorphisms in one patient increases risks for developing neurodegenerative diseases despite the family history of CVDs.

V. Khammad: None. **E. Khammad:** None. **N. Zhuchenko:** None.

E-P15.03**Analysis of association between *TPMT*, *COMT* and *ABCC3* variants and cisplatin ototoxicity in Russian patients**

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Introduction: Cisplatin is a widely used chemotherapeutic agent for the treatment of many cancers. However, its use is restricted by the high incidence of irreversible ototoxicity associated with cisplatin (more than 60% of children receiving it). Some studies have reported that genetic variants of *TPMT* (rs12201199), *COMT* (rs4646316), and *ABCC3* (rs1051640) are conferring increased risk of developing cisplatin-induced hearing loss. However in other studies the results were not replicated. The aim of this study was to replicate the previous studies in an independent cohort of Russian patients. **Materials and Methods:** Total of 45 patients in our study received treatment in pediatric oncology units and had course of cisplatin-based chemotherapy. Among them 16 patients developed hearing loss. SNP genotypes for rs12201199, rs4646316 and rs1051640 were determined in DNA samples of patients, using multiplex ligation-dependent probe amplification. The Fisher exact test was used to determine P values. **Results:** Association between *TPMT* (rs12201199), *COMT* (rs4646316) or *ABCC3* (rs1051640) variants and hearing loss was not observed in our cohort. **Conclusions:** Our results didn't confirm suitability of *TPMT* or *COMT* or *ABCC3* genotyping to identify patients at risk of cisplatin-induced ototoxicity. More researches are necessary, including genomic studies. Therefore, replication of

findings is very important in pharmacogenetic studies. **Table:** Genotype number (and genotype frequency) of *TPMT*, *COMT* and *ABCC3* variants among hearing loss children and normal hearing controls received cisplatin-based chemotherapy

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E-P15.04**Introduction of the nutrigenetics research in Bulgaria shows high genetic predisposition to lifestyle disorders**

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Background: Nutrigenetics and nutrigenomics are intensively developing scientific fields, studying the influence of nutrition on human wellbeing at genetic and genomic levels. The increasing frequency of the lifestyle-related diseases and the personalized treatment approach, leads to usage of genetic testing to personalize a diet plan.

Materials and Methods: A set of 45 single nucleotide polymorphism (SNPs) in 40 genes, related to lipid metabolism, absorption and fats storage, insulin sensitivity, inflammation, oxidative stress, detoxification, osteoporosis, methylation and food responsiveness were studied by the QuantStudio 12 K Flex Real-Time PCR System in 20 Bulgarian probands.

Results: The mean age of the probands is 32,8 with gender ratio 17:3 female to male. After holistic analysis of the SNPs results, was found that each proband is predisposed to at least one social-significant disease, but 12 of them show genetic risk of three or more lifestyle disorders (predominantly atherosclerosis, osteoporosis and insulin insensitivity). Two probands have a high risk for hemochromatosis development, seven - for adult lactase deficiency and three are carriers of *COMT* 472 G > A (catechol-o-methyltransferase) polymorphism, requiring a change in consumption of catecholestrogens and dose reduction of certain catechol drugs. All probands received recommendation regarding the diet, physical activity and surrounding hazards. The follow-up is in progress.

Conclusion: This small, but growing cohort is the first report among Bulgarians, using a personalized approach in the nutritional and daily habits optimization, based on their genetic background and personal gene-diet interactions, affecting nutrient metabolism and transport. This allows individualization in the preventative medicine.

Acknowledgements: DNALIFE ©

SNP	Genotype	Patients with hearing loss (n = 16)	Controls (n = 29)	P value
COMT (rs4646316)	T/T	1 (0.06)	1 (0.04)	p = 0.5882
	T/C	4 (0.25)	7 (0.24)	p = 0.5901
	C/C	11 (0.69)	21 (0.72)	
TPMT (rs12201199)	T/T	0 (0)	0 (0)	
	T/A	2 (0.12)	2 (0.07)	p = 0.4482
	A/A	14 (0.88)	27 (0.93)	
ABCC3 (rs1051640)	G/G	0 (0)	0 (0)	
	G/A	4 (0.25)	10 (0.35)	p = 0.3789
	A/A	12 (0.75)	19 (0.65)	

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E-P15.05

Are vesicular monoamine transporter2 genotypes related to eating behavior and obesity?

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Introduction: Obesity is a health problem which is increasingly becoming prevalent in the worldwide and risky for several diseases. Changes in dopamine neurotransmission affect the brain reward system in a direct way. Furthermore, changes in the reward system influence the eating behavior in human beings and animals. VMAT2 transporter proteins terminate DA function by moving into vesicles in dopaminergic neurons. **Materials and Methods:** In this study, the control group which includes 214 individuals and obese group that involve 234 subjects were investigated for VMAT2 (rs363399 and rs4752045) polymorphisms. Touchdown PCR technique was used for the amplification of VMAT2 polymorphisms. The PCR products of rs363399 and rs4752045 were cleaved by using MspI, and AciI restriction enzymes, respectively. The restriction products were determined by electrophoresis using a 5% agarose gel which was stained with ethidium bromide. **Results:** When the groups were compared in terms of eating behavior, the number of the subjects who ate for reward was significantly higher in obese group ($p = 0.03$). According to the statistical analysis, the subjects who did regular exercise were significantly higher in control group ($p = 0.02$). **Conclusions:** Our findings demonstrate that eating behavior may affect the development of obesity. Moreover, doing regular exercise might be protective for adult obesity. There was no association between VMAT2 genotypes and obesity. It requires further studies in order to understand the relationship between VMAT2 genotypes and obesity. **Key words:** Obesity, dopamine, VMAT2, polymorphism, eating behavior **Grant reference:** This research was funded through a grant from Yeditepe University.

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E-P15.06

Hacettepe University Zebrafish Research Laboratory: Rare diseases modeling in zebrafish by using genome editing tools

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Hacettepe Medical Center is the largest JCI accredited hospital outside of North America, serving about 30 million patients. It is estimated that 7 million people are affected by rare diseases in Turkey. Families with tentative diagnosis of rare diseases are referred to Hacettepe Rare Diseases Biobank holding 35.000 DNA samples and 1.200 cells/tissues from 5.800 families with neurological (46%), metabolic (19%), nephrological (15%), gastroenterological (9%), immunological/haematological (5%), chest diseases (3%) and dermatological disorders (3%). This extensive bioregistry facilitate discovery of novel genes, diagnostic and prognostic biomarkers, and therapeutic research. Long child bearing age, multiple affected families and observation of new phenotypes with unknown genes is an added value for research and spectra of clinical phenotypes makes hunting for modifiers more approachable. We used these availabilities as an advantage and established a Zebrafish Research Laboratory. Our aim is to create patient-specific disease models by using genome editing tools in zebrafish in order to understand underlying mechanisms of diseases. Muscle disorders and corneal dystrophies are ongoing research subjects. Also we generate knockout models to test candidate ultra-rare genes identified by our university researchers revealed by exome sequencing. Furthermore, we are planning to form a catalogue of mutant zebrafish stocks consisting of specific mutations that we hope will contribute to future studies of drug and treatment. This study was funded by TÜBİTAK Project number 214S174. We thank Prof. Meral Özgür (Hacettepe Rare Diseases Biobank) for her contributions.

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E-P16 Omics/Bioinformatics

E-P16.01

Functional analysis of the mutated actin to study its effect on congenital myopathy

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Introduction: Congenital myopathy is a broad category of muscular diseases which its symptoms appear at the time of birth. One of the types of congenital myopathy is nemaline myopathy which is characterized as a disease with variable range of severity. Nemaline bodies are observed in the biopsy samples. M46T in actin is one of the causes of this kind of myopathy. M49 is located in the D-loop which is responsible for the stability of the F-actin by wrapping around Tyrosine 171 of the adjacent G-actin and fit itself in the hydrophobic groove of it. To our knowledge, the effects of this mutation have not been studied yet. **Materials and Methods:** Wild and mutated types of actin was modelled using EasyModeler2.1. The structure of mutant is compared with wild actin protein. Conformational changes were simulated using GROMACS 4.6.3 software package for 100 ns. **Results:** It was realized that M46T changes the structure of D-loop in a way that D-loop is no longer able to be fitted properly in the hydrophobic groove. Moreover, Threonine is more polar and hydrophilic than Methionine which affects the polarity of the D-loop and weakens the hydrophobic interaction between two adjacent G-actins. **Conclusion:** These changes seem to be related to the abnormal assembly of the F-actins which result in the nemaline myopathy.

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E-P16.03

oncoIMGViz: bioinformatics cancer analysis platform for NGS genes panels

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Introduction: oncoIMG_Viz is a NGS bioinformatics production platform for analysis of cancer targeted genes panels using paired samples (germlinal-somatic). **Material and methods:** The pipeline is based in open source genomic software like bwa, samtools, picard, strelka,

freebayes, MuTect, VarDict, Lumpy and our own package oncoIMG_CNV for the data and MGviz and jviz for the web visualization. **Results:** The tool provides to clinicians an interactive tool for data management and visualization for filtering somatic variants yielding the right set of quality control annotations for filtering out dubious SNPs and helps to discriminate subclonal variants at different levels of variant allele fraction (until 5%). The tool also helps in the prioritization of variants providing standard variation germinal annotation and adding annotations of cancer pathogenicity, LOH, and known drug interactions. The tool calculate and visualize the CNVs and use them to improve the genotype interpretations.

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E-P16.04

Global genetic/clinical databanks are crucial for high-quality interpretation of WES/WGS data - CentoMD® as a general reference platform in genetic diagnostics

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Background: The majority of the existing reference databases (DB) is not built on stringent quality, multi-ethnic populations, standardized annotation of the patient phenotypes, and sometimes do not follow evidence-based standards during variant classification. CentoMD® is a browser-based tool that enables access to a high-quality repository of genetic and human phenotype ontology (HPO)-based clinical information. **Methods:** Data are gathered following a case-oriented model, where patient information, standardized HPO clinical data, testing methodology and detected genetic variants are compiled together. Curation process is divided in three phases: variant-wise, case-wise and warnings-wise procedures. All patients provided informed consent before inclusion in the DB. Once included, all patient data are fully anonymized. The classification of genetic variants is done according to ACMG guidelines. Detected genetic variants are first classified into one of three classes: clinically relevant variants (CRV); clinically irrelevant variants (CIV); and variants of uncertain significance (VUS). **Results:** The patient cohort (> 115,000 genetically screened individuals in CentoMD® v3.2) is highly heterogeneous covering the global population (> 110 countries worldwide). The observed age at genetic diagnosis ranges from prenatal stage to > 80.0 years. More than 660 million

variant detections are included. Approximately 55% of the CRV and VUS variants are novel. Notably, >3% of the genetic variants previously reported in the literature as being pathogenic, were reclassified based on strong internal evidence as clinically irrelevant. Conclusions: CentoMD® brings exceptional quality, unique and valuable information on genetic variants spectrum in different ethnical populations, even in those under-represented in other public DB.

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E-P16.05 A Critical SNP of AXIN1 in Colorectal Cancer Bioinformatics study

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Colorectal cancer(CRC) after skin, breast and stomach cancers is the most prevalent cancer. The cause of this disease is unknown, but the results indicate environmental and genetic factors play important roles in its creation. Mismatch in repair of unpaired nucleotides in genes can also be seen in CRC. The Wnt/β-catenin is an important cell signaling pathway in intestinal homeostasis and usually is unregulated in CRC. Axin1 is an important component of this pathway and a negative regulator of Wnt/β-catenin signaling, which interacts with some proteins in β-catenin degradation complex. Mutations in this gene increases the risk of CRC therefore the knowledge of these changes can play an important role in the early detection or treatment of CRC. In this paper using novel bioinformatics approaches we determined the nucleotide sequence of exon5 of axin1 which could be mutated and was selected as one of the most important SNPs(nucleotide70 G/T and position 304372 with rs34440193). Then using the Swiss-Model software we predicted the tertiary structure and using Chimera and Deep View Molecular Viewing softwares the importance of mutation in this area was studied at their protein level. Mutation in this area causes a change in leucine which strictly is aliphatic amino acid to Methionine and changes the electrostatic forces, bond distance, overall Protein energy and Reduces its stability. When axin1 cannot do its

activities properly, Reduced tumor inhibitor activity. therefore, β-catenin is destabilized and this can help Cancer progression along with the expression of major related genes. Our bioinformatics study results can be verified using laboratory methods.

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E-P16.06

Copy number estimation from targeted and shallow sequencing in cancer samples

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Introduction: Next-generation sequencing (NGS) is mainly used to obtain sequence variants (SNVs). However, obtaining copy number results from NGS has gained momentum in both research and clinical applications, using whole genome, whole exome and targeted sequencing panels. Materials and Methods: Here we introduce the BAM (MultiScale Reference) algorithm to function with shallow and targeted sequencing data, as well as WGS and WES, using a novel dynamic binning approach. This approach uses a Hidden Markov Model to segment the genome into target areas using the reads in targeted regions and the backbone areas using the off-target reads and additional areas. Results: Shallow WGS data and targeted panel NGS data, as well as WES with normal depth of coverage, were used for the testing. The results were compared with those from microarray, BAM ngCGH (matched) and BAM (pooled reference). Differences in overall read-depth resulted in variable sample quality across the cohorts, however most sample quality was adequate for copy number estimation and a quality threshold was assessed. Results indicate that relative copy number can be estimated and is comparable to the results achieved with microarray for the same targeted regions. Conclusion: The BAM (MultiScale Reference) method has been tested in a variety of cancer samples. This is an ideal tool for copy number estimation with NGS results in cancer samples because it provides a way for non-matched-pair analysis with genome, exome and targeted NGS.

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E-P16.07

F1174V Mutation Alters the ALK Active

Conformation in Response to Crizotinib in NSCLC: Insight from Molecular Simulations

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Crizotinib is an efficient antineoplastic drug for treatment of non-small cell lung carcinoma (NSCLC), which is identified as an anaplastic lymphoma kinase (ALK) inhibitor. F1174V is a recently identified acquired point mutation relating to the Crizotinib resistance in NSCLC patients. The mechanism of Crizotinib resistance relating to F1174V mutation as a non-active site mutation remains unclear. In this study, the molecular dynamic simulation was used to investigate the possible mechanisms by which F1174V mutation may affect the structure and activity of ALK kinase domain. The results suggested that F1174V mutation could cause two important secondary structure alterations, which led to the local conformational change in ALK kinase domain. This causes more positive free energy in the mutant complex in comparison with the wild-type one. In addition, our structural analyses illustrated that F1174V mutation could result in some important interactions, which represent the key characteristics of the ALK active conformation. This study provided a molecular mechanism for ALK Crizotinib resistance caused by F1174V mutation, which could facilitate designing more efficient drugs.

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E-P16.08 Genome annotation and assembly assessment in Ensembl

A. Zadissa, W. Akanni, B. Aken, J. Allen, J. Bhai, S. Boddu, F. Martin, M. Muffato, D. Murphy, D. Sheppard, H. Riat, P. Flicek, A. Yates

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With the rise of long-read sequencing technologies and ever reducing costs for genome sequencing, there has been a significant increase in the number of genome assemblies that could be added to Ensembl. In many cases these genomes have already been annotated. Key to incorporating these resources is a quick and robust assessment of their characteristics with respect to those already supported by the Ensembl project. This process will inform the methodologies required to integrate, distribute and present the data in a consistent manner. It will also determine whether the protein-coding and non-coding gene sets are suitable to be added to our gene orthology annotation. Our methods are

capable of assessing a wide range of assemblies and annotations of varying completeness. We have used our existing data sets to define assessment criteria and categories. Specifically, assemblies are assessed against criteria such as similarity to closely related genomes and we evaluate annotations using reference genomes as a benchmark. In addition we have evaluated a subset of non-vertebrate assemblies from our sister project Ensembl Genomes. This includes a number of species with significant impact on human health. We will describe our methodology, results from a selection of the vertebrate and non-vertebrate assemblies and report on how these data will be made publically accessible and displayed.

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E-P16.09

Structural and functional analysis of a novel mutation in human pyrin B30.2 domain in Azerbaijan

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Introduction: Familial Mediterranean fever (FMF) is a recessively inherited autoinflammatory disorder that is frequently carried by different populations from eastern Mediterranean basin. The disease is caused by mutations in MEFV gene that is located on chromosome 16p13.3 and encodes pyrin. Around 180 mutations have been reported to associate with FMF, while the five most frequently encountered mutations are M694V, V726A, E148Q, M694I, and M680I that are considered as origin for 74% of FMF mutations. Four mutations within this set are located on the B30.2 domain in Pyrin protein. **Materials and Methods:** The effect of a new FMF-associated mutation Q753R was investigated that is located on β 13 in the B30.2 domain. The dynamic behavior of this new mutant was compared with four most commonly seen mutations in this domain using the GROMACS software package. **Results:** The results indicate that the overall structure of the mutated proteins is highly different from the native protein. These differences cause to increase the instability of the domain. Furthermore, studying the dynamic behavior of the proteins reveals that RMSD of the mutated structures is remarkably

increased while radius of gyration is different from the native structure during MD simulation. In addition, the number of H-bonds is increased in all mutants demonstrating an increase in rigidity of the mutated structures. Conclusion: In summary, based on the MD simulation results, Q753R mutation has a destabilizing effect on B30.2 domain of Pyrin and is a pathogenic disorder similar to four other mutations in B30.2 domain of pyrin.

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E-P16.10
Application for genomic variant annotation, filtering and prioritization

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Introduction: Analyses based on massively parallel sequencing detect vast amounts of variants, while only few are responsible for traits of interest. Successful identification of these few variants requires annotation with various features, especially function prediction score and conservation score. Different annotation data is scattered across various databases, which makes manual annotation a time-consuming and tedious process. Methods: To facilitate the annotation process, we developed a desktop application called Variant Annotation Analyzer (VAA) together with web based Variant annotation service (VAS). VAA relies on VAS for automatic annotation. The VAS acts as front-end for dbNSFP database which aggregates many of variant databases. We have also developed prioritization process based on user preference. Results: VAA supports automatic annotation of variants in VCF file format. Annotated variants can be further filtered, sorted, prioritized and exported. Modular architecture based on plugins allows easy implementation of new features and additional annotation providers. Conclusion: The VAA application and the VAS web service provide researchers with rich and automated annotation of variants in a fraction of time compared to manual annotation. With the option of further prioritization it provides a powerful tool for fast identification of potential candidate mutations among loads of irrelevant variants. The use of the web service is not limited to the VAA application and is fully open to any academic use.

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E-P16.11

France Génomique: a national research infrastructure that brings together French expertise and equipment for genomic analysis and bioinformatics

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France Génomique (FG) is a national genomics infrastructure born out of a desire to reinforce and optimise French capacity in the field of high-throughput genomics and associated bioinformatics. In the context of strong international competition, the prime objective of FG is to maintain France at the highest level of competitiveness and performance, at the cutting edge of genomics data production and analysis, thus reinforcing France's visibility in the international genomics landscape. The FG infrastructure brings together the majority of the French sequencing and bioinformatics platforms, incorporating significant capacities in genomic analysis and bringing together the critical mass and expertise necessary to enhance the competitive strength of the French scientific community and attract large international projects. FG draws on a pool of recognised and complementary expertise, placed under integrated governance, to offer coordinated access to a range of know-how covering all areas of the life sciences (biodiversity, medicine, agronomy, etc.). In addition, its coordinated technology development programs enable FG to provide the scientific community with access to the newest state-of-the-art technology and expertise internationally available for high throughput sequencing and data processing. Thus FG operates both as a developer of innovative approaches to constantly offer the most competitive services, and as a collaborator to the scientific community for projects submitted either to an annual call for proposals for large-scale projects, or to a continuous submission scheme on the FG web portal for smaller-scale projects. FG was created thanks to an "investissements d'avenir" grant from the French government, ref. ANR-10-INBS-0009

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E-P16.12

Expediting the availability of data in the GWAS Catalog

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The genome-wide association studies (GWAS) Catalog (<http://www.ebi.ac.uk/gwas/>) is a publicly available, manually curated repository and visual representation of all published human GWAS with their SNP-trait associations. The Catalog contains data on over 35,000 associations from over 2,700 publications, covering a wide range of human diseases and traits in an expanding number of ancestral backgrounds. The volume of eligible data continues to grow which has led to delays between journal publication and inclusion in the Catalog. To improve the Catalog for our users we have been investigating methods of expediting data availability. We have carried out a pilot of author submission to investigate whether authors can provide structured information directly to the Catalog, increasing the efficiency of inclusion in the Catalog. The participants were able to submit their results to a prototype deposition interface through online forms. The feedback was that the interface design was easy to understand and resulted in a prompt submission of all relevant data with high level of accuracy. Currently eligible studies are prioritised chronologically, however we are investigating alternative strategies to ensure that data with the highest relevance and utility to users would be available quicker. This could include prioritising studies based on the user's queries, downloads, and searches. To avoid bias, in addition to traits, we will consider the number of samples analysed, in addition to ancestry. We welcome feedback on these changes to the Catalog. Funding: NHGRI, NHLBI, the NIH Common Fund (U41-HG007823 and U41-HG006104) and the European Molecular Biology Laboratory.

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E-P16.13 **Integrative approach to biomarker discovery by performing comparative analysis of two cancers Hepatocellular carcinoma and Endometrioid endometrial carcinoma using genetics and transcriptomics from RNA sequencing data**

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Hepatocellular Carcinoma (HCC) and Endometrioid Endometrial Carcinoma (EEC) are two lethal diseases of public health importance worldwide. Using QIAGEN's RNA sequencing solution, we were able to highlight key molecular and biological processes that indicate similarities in the tumor progression toward metastasis between one EEC patient and the three HCC patients. We have identified ITGB1-001 isoform as a potential common biomarker between these two complex cancers. We have also shown these two cancers share activated pathways such as actin cytoskeleton signaling, and RAF1 as an upstream regulator indicating similar transcriptional program between these patients. Furthermore, we have identified as a potential therapeutic target the master regulator CTGF that connects a network of 286 downstream targets to invasion of tumor cells. We have filtered a set of unique variants in the EEC patient that affect a network of upstream regulators common with HCC. This approach may be useful in the context of precision or personalized medicine. Examining the gene expression in tumors from groups of patients with each disease revealed that at a molecular level, early stages of EEC resemble established HBV-positive, HCV-negative, liver cirrhosis positive HCC.

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E-P16.14

Insights from indels profiling in a cohort of 123 Brazilian patients with congenital malformations and neurological disabilities

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Over the years, the newer methodologies and analytical procedures for arrays experiments became even more refined and precise. This evolution, mainly in bioinformatics, offered innovative tools for classifying newer or existent copy number variations reports into more precise categories and diminish the VOUS account. We applied these tools on cytogenomics data of a cohort of 123 patients from Cytogenomics Laboratory of FMUSP in order to find common types and subtypes of CNVs in Brazilian population. No gender or age was discriminated in this analysis. The data of 123 patients were collected from multiple SNP-arrays experiments from different platforms. Bioinformatics techniques were used to classify CNVs into fitting categories and statistics were calculated with RStudio (1.0.136). Only duplications and deletions were considered. Among the 123 patients, 109 CNVs consisted of deletions and 160 CNVs were duplications, some of them concomitants. Also 135 indels were classified as pathogenic, 107 benign and 27 VOUS. The chromosomes X, 9, 22 and 7 showed the most number of alterations: 39 (14.5%), 24 (8.9%), 23 (8.6%) and 21 (7.8%), respectively. Our study revealed that Brazilians patients present more duplications than deletions, which, in accord to DGV, is not the same in other populations. And the most affected chromosome is the X. The refinement of bioinformatics tools and the help of databases of CNVs made the classification easier and more precise, letting researchers evaluate more data in less time with more precision and confidence in different populations. Grants: FINEP-CT INFRA 0160/12 SP8, CAPES

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E-P16.15

High-throughput protocols for fast 16 S rRNA-gene based metagenomic analysis with MiSeq and Ion Torrent PGM systems

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Introduction: Next-generation sequencing targeting the 16 S rRNA gene, a common procedure to quantify and classify bacterial species from environmental samples, has been fruitfully utilized to detect dysbiosis associated with human disease and treatment responses. Here we aimed to establish fast and straightforward experimental and analytical procedures for its application in clinical settings.

Methods: Starting from genomic DNA, five consecutive steps are performed for 16 S rRNA amplicon library preparation, including PCR, bead-based purification and fragment size selection, mass-normalization, pooling and quantification. A customized bioinformatics pipeline was designed to facilitate the integrated use of common filtering steps as well as for multipurpose statistical analysis.

Results: Here we present a sample-to-answer protocol to facilitate library preparation as well as sequence data analysis and interpretation compatible with both Illumina and Ion Torrent technologies. The complete process for preparing 96 libraries in parallel can be achieved in half a day involving less than 1.5 h hands-on time. Performance of the analytical procedures for the MiSeq and Ion Torrent PGM platforms was evaluated on libraries obtained from a reference bacterial DNA mock community by assessing bacterial abundance and diversity.

Conclusions: The proposed protocol is able to determine the expected bacterial diversity at genus level, requiring minimal training from the user.

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E-P16.19

jviz webcomponents: a foundation js framework for clinical NGS bioinformatics

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Introduction: MGviz is a fully integrated NGS QC and data analysis suite that creates automatic NGS clinical reports for clinicians to review. Summarized quality control measures with threshold based from all the previous runs are computed to warn about any kind of bias and check also for sample swap. These are Intuitive and interactive tools that help prioritize and select the variants of interest related to the patients phenotype from our NGS gene panels and commercial exome panels. **Materials and Methods:** We have created javascript webcomponents that can be easily integrated in web applications. Each component has his own API reference described in the jviz GitHub. These components can import the data from an associated RESTful service, and achieve great interactivity by means of the use of HTML5 canvas element as a drawing mechanism. **Results:** A suite of independent webcomponents, that intercommunicate via events give them great modularity and strength. Also, it allows the easy integration of several of them in fully functional web applications for quality control of the NGS analysis and the variants prioritization for the genetic diagnosis. **Conclusions:** We have also developed a full suit of open source tools in python, R and MEAN stack for creating clinical bioinformatics as a service. All this is bases in our own modern js library for interactive bioinformatic visualization: jviz (<https://github.com/jviz>). This study an JMJ has been funded by Instituto de Salud Carlos III “IFI15/00138” (Co-funded by European Regional Development Fund/European Social Fund).

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E-P16.20

Clinical usefulness of genotype-driven and genotype-and-phenotype-driven variant prioritization algorithms (GAs and GPAs) for whole genome sequencing (WGS) analysis

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Background: Variant prioritization algorithms are an increasingly used approach for clinical WGS analysis of rare disease (RD) patients. Recently, algorithms extending GAs by including phenotypic information to improve accuracy - GPAs - were introduced. Here, we compare the performance of a GA and two GPAs in identifying likely disease-causing variants found in patients from our WGS cohort (HICF2) to test the methods’ clinical validity.

Methods: One GA (VAAST) was compared with two GPAs (Exomiser-v7.2.1, VAASST + Phevor) using WGS data from seven RD patients. The cases included singletons and trios, different inheritance patterns (autosomal/*de novo* dominant, autosomal/X-linked recessive) and known and novel gene candidates. Phenotypic data was described using the Human Phenotype Ontology. Strong candidate variants, identified manually by human disease experts with our clinical analysis pipeline, were used as benchmarks to test the algorithms’ effectiveness in prioritizing causal variants.

Results: The algorithms ranked the benchmark variants in the top eleven for all seven cases. Phevor + VAASST ranked five benchmark variants first, followed by VAASST (4/7) and Exomiser (3/7). While the GPAs did not significantly increase the number of benchmark variants ranked first, they reduced the number of high-scoring variants requiring further analysis, saving time and costs. Particularly for a consanguineous case with many homozygous variants, the GPAs accelerated the analysis, downgrading variants unrelated to the phenotype.

Conclusions: Our results demonstrate the usefulness of ranking algorithms for analysing clinical RD cases, while also making a case for collecting structured phenotypic information to improve clinical workflows. **Acknowledgments:** Funded by HICF2 and WTCHG Grant 090532/Z/09/Z.

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E-P16.21**Bring Your Own Data workshop is an excellent tool to promote the establishment of Findable, Accessible, Interoperable, and Reusable rare disease registries**

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Increasingly, Rare Disease (RD) registry managers are realizing that poor interoperability and access restrictions keep their data siloed and underexploited. Registries should strive to be more Findable, Accessible, Interoperable, and Reusable for humans and computers (FAIR), to conform with emergent global standards. To promote the establishment of FAIR RD registries, the National Centre for Rare Diseases, Istituto Superiore di Sanità, Rome, Italy (CNMR-ISS) and DTL/ELIXIR-NL have organized ‘Bring Your Own Data’ workshops (BYODs) for RD registry managers since 2014: a hands-on learning experience where RD (data) experts and Linked Data experts collaborate to enable integrative questions across individually FAIR registries. We present acquired insights and the organisational roadmap of these BYODs, encompassing (i) a preparatory phase with two webinars for registry managers, (ii) the two-day BYOD, (iii) a follow-up phase to foster the results of the BYOD. The friendly environment of a BYOD stimulates open information exchange between experts from widely different disciplines. Registry managers experience how preparing data for linking *at the source* makes difficult queries easy. They learn about the requirements to make a registry FAIR and their role as managers. Since the first BYOD, we shifted focus from data only to managerial advice. We see a growing interest, positive feedback, and follow-up in the areas covered by the BYOD. We greatly thank organizers, and all participants for their essential contribution to the success of the BYODs, that have been supported by DTL, Elixir, Elixir-Excelerate, CNMR-ISS and RD-Connect. CC, MR, MJ equally contributed to the work.

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E-P16.23**Normalization and correction for batch effects via RUV for RNA-seq data: practical implications for Breast Cancer**

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Introduction: The whole transcriptome contains information about nonsense, missense, silent, in-frame and frameshift mutations, as observed at whole-exome level, as well as splicing and (allelic) gene-expression changes which are missed by DNA analysis. One important step in the analysis of gene expression data arising from RNA-seq is the detection of differential expression (DE) levels. Several methods are available and the choice is sometimes controversial. For a reliable DE analysis that reduces False Positive DE genes, and accurate estimation of gene expression levels, a suitable normalization approach (including correction for confounders) is mandatory. **Materials and Methods:** Several normalization methods have been proposed to correct for both within-sample and between-sample biases. RUV (Removing Unwanted Variation) is one of them and has the advantage to correct for batch effects including potentially unknown unwanted variation in gene expression. In this study, we present a comparison on real-life paired-end sequencing data for ER + Breast Cancer tissues versus matched controls between RUVg (using *in silico* negative control genes) and more commonly used methods for RNA-seq data normalization, such as DESeq2, edgeR, and UQ. The set of *in silico* empirical negative control genes for RUVg was defined as the set of least significant DE genes obtained after a first DE analysis performed prior to RUVg correction. **Results and conclusions:** Box plots of relative log expression (RLE) among the samples and PCA plots show that RUVg performs well and leads to a stabilization of read count across samples with a clear clustering of biological replicates. **Grant:** WALInnov-NACATS 1610125. **A. Debit:** None. **S. Wenric:** None. **C. Josse:** None. **V. Bours:** None. **K. Van Steen:** None.

E-P16.24**Genotyping of short tandem repeats**

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Introduction: Short tandem repeat (STR) is an uninterrupted repeat short stretch of DNA, typically with a 2–10 nucleotides repeat unit. Number of repeats varies widely in population which makes it a suitable tool for forensic analysis. In addition, several genetic diseases are associated with extreme prolongation in exonic regions, such as CTG motif exceeding 50 repetitions in DMPK gene is known to cause myotonic dystrophy. **Materials and methods:** We propose algorithm for identification of fragments originating from STR locus of interest. The method accounts for natural deviations from expected sequence, such as variation in repeat count, sequencing errors, ambiguous bases and conjunction of several basic motifs. In addition we implemented a calibration of aggregated allele counts to correct for copy number defects caused by the polymerase induced stutter effect. **Results:** We designed genotyping tool for STR motifs, called Dante. Sequenced DNA fragments are profiled against motifs of interest that can be defined in a configuration file. Allele genotypes with associated call confidences are reported in a form of summary table. **Conclusions:** Precise genotyping of STR alleles from DNA fragments is useful addition to existing NGS data analysis pipelines to allow interpretation of STR loci. We demonstrate that despite their hypervariability and errors in sequencing process, it can be handled by a complex model presented in the tool Dante.

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E-P16.25

ICELL8™ single-cell system enables the enumeration of paired α and β chain sequence information for T-cell receptors from thousands of cells

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Single-cell RNA-seq has quickly become the method of choice to identify and classify cell types present in complex tissues based on cell-to-cell gene expression differences. A number of different methods exist to process thousands of cells in a single experiment. Low throughput methods such as FACS can sort cells based on phenotypes but is expensive on per-cell basis. None of these methods enable a direct link between observable phenotypes and gene expression differences at a single cell level for thousands of cells per experiment. To address this need, we developed a single

cell system, ICELL8™, that enables researchers to select cells based on fluorescently labeled biomarkers (such as red fluorescent protein or Hoechst/propidium iodide live/dead stain) and further process only the single-cells of choice for genomics applications. The system uses nanowell arrays up to 9600 wells with well-volumes up to 1 µl, which can be flexibly configured to perform multi-step method development at very low costs. Sequencing the variable regions of paired α and β chains of T-cell receptors is a critical need to better understand T-cell biology and its role in immuno-oncology. We have developed a method to identify paired clonotypes of α and β chains from over a thousand cells. Details of this work will be presented using Jurkat cells and peripheral mononuclear blood cells (PBMCs).

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E-P16.26

RD-Connect: distributed elastic architecture for rare disease research

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RD-Connect (rd-connect.eu) is an integrated platform that aims to bridge the gaps in rare disease research. The platform architecture has been designed to use general purpose, open-source software in all layers on which the platform is built, seeking to take advantage of the latest development in computer science while ensuring scalability. The platform requires centralised authentication, real-time access to combined -omics data and phenotypic information, interoperability with catalogues of resources, and communication with international initiatives such as the GA4GH Beacon project and the GA4GH/IRDiRC Match-Maker Exchange. To fulfill these requirements, a mix of technologies and applications are deployed in an

environment using Foreman and Puppet to provision and manage the hardware layer. Most physical resources are managed through DCOS (based on apache mesos), which abstracts all the resources (CPU, memory and storage) away from machines, enabling a fault-tolerant and elastic distributed system (kernel layer). Many of the applications are running inside docker containers, orchestrated by Marathon. To cater for different data access requirements, we use a set of differentiated solutions. Hadoop hdfs was chosen for high throughput processing, elasticsearch for low latency queries, and RDBMS (postgres, mysql) for ACID transaction requirements. The Extract-Transform-Load (ETL) pipeline is used to parse genomics data, consolidate sample information by genomic coordinate, enrich the data, and load the product into elasticsearch. ETL jobs are leveraged by apache Spark allowing distributed processing. Finally, Jenkins is used for automation of all tasks, from data transfer to pipeline running and service deployment, including testing and validation.

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E-P16.27

tet(M) gene sequence typing from tetracycline-resistant *Ureaplasma parvum* and *Mycoplasma hominis* isolated from tunisian patients

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tet(M) gene sequence typing from tetracycline-resistant *Ureaplasma parvum* and *Mycoplasma hominis* isolated from Tunisian patients Nadhem Aissani, Boutheina Mardassi Unit of Mycoplasmas, Pasteur Institute, Tunis, Tunisia Resistance to tetracyclines in genital mycoplasmas is due mainly to acquisition of the tet(M) determinant, which is frequently associated with conjugative transposon elements of the Tn916/Tn1545 family. The aim of the present work was to evaluate the prevalence of tet(M) in Tunisian isolates and to gain an insight into its origin and evolution. Twenty *Ureaplasma parvum*, two *Ureaplasma urealyticum* and 48 *Mycoplasma hominis* isolates, recovered from Tunisian patients with urogenital and infertility disorders, were evaluated for their resistance to tetracyclines and interrogated by PCR amplification for the presence of tet(M) and int-Tn. The resistance rates to tetracyclines were 22.72 and 25% among *U. parvum* and *M. hominis* isolates, respectively. All resistant isolates harboured both tet(M)

and int-Tn sequences. Molecular typing indicated that the tetracycline-resistant *U. parvum* and *M. hominis* isolates were not clonal. Taken together, these data indicate that a single tet(M) gene sequence type, most probably transmitted via a Tn916/Tn1545-like transposon, contributes to most of the tetracycline resistance in *U. parvum* and *M. hominis* isolates in Tunisia. Because this tet(M) gene sequence type was harboured by different *Mycoplasma* spp. and by phylogenetically distinct isolates within these species, one could reasonably argue that it may have benefited from an efficient horizontal transfer context, making it highly competent to spread.

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E-P16.28

The added value of SNPs - user friendly applications for CytoScan Cytogenetics Suite trio-analysis

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Introduction Microarray analysis has been generally accepted as the first tier test for the detection of CNVs in e.g. patients with intellectual disability. The use of SNP-based arrays will not only allow the detection of CNVs, but also generate genotyping information. This "extra" information can be used to increase diagnostic yield and to assure test quality. Increasing the diagnostic yield can be achieved by uncovering uniparental disomies (UPDs), regions of homozygosity (ROH), parental origin and allelic imbalances. Here we present applications to analyze CytoScan array data (Thermo Fisher Scientific) for rapid detection of such uniparental disomies (UPDs) and parent of origin annotation of de novo deletions and duplications. Validation and performance data is also presented. Materials and Methods The development of the applications was based on using the genotype-data from patients with known genetic aberrations. This resulted in two windows executables that do not require prior installation, MyUPDFinder and MyPODFinder. Results MyUPDFinder was primarily designed to detect UPDs and parental origin of de novo deletions, whereas MyPODFinder detects parental origin of de novo duplications. Samples with known relationships and aberrations were used for validation. New findings include cases with complete uniparental heterodisomy. Conclusions MyUPDFinder and MyPODFinder are suitable for SNP duo/trio-analysis. By using these tools we can

increase our diagnostic yield of array diagnostics by detecting e.g., partial or whole chromosome UPDs and parental origin of de novo deletions or duplications. The applications also provide assurance of trio-consistency and absence of sample mix-ups or non-paternity.

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E-P16.29 **An Integrated clinical variant confirmation tracking and primer designer system**

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With the current increase in genetic and cancer diagnostic screening, there has also been an increase in demand for PCR based confirmations of identified variants. The increase is due to large scale genome projects like the DDD and 100,000 genomes project, and the education of the general public regarding the availability of these tests. With the increased number of confirmations (~100/month) being performed in our laboratory the previous method of manually designing primers and tracking confirmations is no longer feasible in a high throughput environment. Here we present a web-based confirmation system that can be run on a standard computer. Alternatively, the primer designer part of the system can be run on its own if needed, outputting either text or PDF reports. The system allows the users to setup confirmations; entering patient and variant information, and then track the whole process from selecting primers, tracking primer orders, ongoing confirmations and the reporting of the confirmations. When selecting primers for a confirmation the user is informed if any of the primers have already been purchased and if any primer combinations have previously worked or failed. Furthermore, when possible, the primer design captures full exons +/- 10 bp of the introns to ensure that the primers can be reused while also ensuring the primers do not contain any common SNPs. Finally, in the primer selection phase all primer pairs are analysed for pairs giving multiple products. All of these contribute to significant time and cost savings for the laboratory.

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E-P16.30 **A new method for analysis of whole exome sequencing data (SELIM) depending on variant prioritization**

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Introduction: After the first genome had been sequenced in 2003 with an international project, Human Genome Project, the 1000 Genomes Project also revealed the analysis of 1092 and 2504 genomes respectively. Whole exome sequencing of human samples was reported to detect approximately 20,000–30,000 SNV and indel calls on average. It is very important to choose the best tool that suits the related study. **Materials and Methods:** In this study, it is aimed to demonstrate the results of an in-house method (SELIM) for variant prioritization of WES data without using in-silico methods. **Results:** By this method, the annotated data have been decreased by 7.4–13.8 times (mean = 10.9). **Conclusions:** By the initiation of 1.000.000 genome project, powerful databases are needed. In this respect, SELIM is an in-house workflow that can easily be used for simplifying the annotated data without using any in-silico methods.

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E-P17 Epigenetics and Gene Regulation

E-P17.01

***IL-4*, *VEGFA* genes and micro-RNAs miR-619 and miR-1273g expression in human leiomyoma compared to normal myometrium**

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Introduction. Uterine leiomyoma (ULM), one of the most common reproductive tract neoplasms in women, is a kind of benign tumor with multigene involved. The formation and growth of these tumors is thought to be, in part, the result of several genetic and epigenetic mechanisms. The dysregulated microRNAs were reported to play important roles in ULM pathobiology by regulating tumor growth. Evidence of irregular miRNA expression in uterine fibroids has garnered recent interest for diagnostic and therapeutic applications. Study was aimed to investigate the expression of *IL-4* and *VEGFA* genes, involved in inflammation and angiogenesis, and miR-619 and miR-1273g, which we have previously identified as a possible target *IL-4* and *VEGFA* genes regulators.

Materials and methods. Forty eight paired tissue samples of uterine fibroids and normal myometrium were obtained from 24 women age 33–69. Expressions of target genes and mi-RNAs were analyzed by real-time PCR.

Results. Our research revealed *IL-4* expression increase in 33.3% and decrease in 50% of samples compared to normal myometrium (fold change > 1.5, $p < 0.05$). Increased expression of *VEGFA* (fold change > 1.5, $p < 0.05$) was registered in 62.5% and decreased in 12.5% of uterine fibroids. Differential expression of miR-619 was identified in 71% fibroids, compared to normal myometrium, wherein in 47% of fibroid tissues we observed decreased expression, and in 24% cases expression was increased. MiR-1273g expression was lower in 75% of ULM samples.

Conclusion. MiRNAs as either indirect or direct regulators of gene expression impact the pathobiology of uterine fibroids.

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E-P17.02

Lipoprotein(a) associates with shorter relative telomere length in pediatric hypercholesterolemic patients

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Introduction: Hypercholesterolemia [HH] is in pediatric population frequently associated with mutations in genes involved in metabolism of cholesterol. Besides the serum cholesterol level, Lipoprotein(a) [Lp(a)] is an independent risk factor for the development of atherosclerosis and other related cardiovascular diseases. Correlation of telomere length [TL] with Lp(a) as a marker of increased oxidative stress and cell replication in risk of atherosclerosis has so far not been assessed. **Materials and Methods:** 26 HH patients with Lp(a) level higher than 500 mmol/L and 32 HH patients with Lp(a) lower than 100 mmol/L, were matched in levels of total cholesterol, LDL, HDL, age, anthropometric data and sex ratio. For every participant relative TL [rTL] were assessed using rTL QPCR method. Data were statistically evaluated using t-test. **Results:** Results indicate statistically longer rTL (1.086 ± 0.04387) in HH patients with Lp(a) lower than 100 mmol/L compared to rTL (0.8937 ± 0.03543) in HH patients with Lp(a) level higher than 500 mmol/L ($p = 0.0013$). **Conclusion:** Results of our study indicate a strong correlation between higher levels of Lp(a) and shorter rTL, which might be the result of an early

inflammation stage of atherosclerosis and cardiovascular diseases. The national screening program is important for detection of HH and prevention of HH with increased risk for complications. Nevertheless, additional biomarkers to assess the risk for arteriosclerosis are of outmost importance. Our data regarding rTL and association with atherosclerosis risk are valuable but need further confirmation. This work was supported by the Slovenian Research Agency grant J3-6800.

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E-P17.03

Analysis of lncRNAs in plasma in breast cancer patients

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Introduction: Long non-coding RNAs (lncRNAs) are defined as RNA molecules longer than 200 nucleotides with poor protein-coding capacity. lncRNAs are key regulators controlling the gene expression. Dysregulation of lncRNAs (e.g. HOTAIR) in tissues was detected in breast cancer (BC). Plasma samples are examined as liquid biopsies, therefore research and potential use of lncRNAs as plasma markers became highly topical. **Materials and Methods:** 84 lncRNAs have been profiled in 15 plasma samples - 6 normal and 9 BC, using Human Inflammatory Response & Autoimmunity RT² lncRNA PCR Array. Total RNA from plasma samples was isolated using miRNeasy Serum/Plasma Kit. Data analysis was performed using qBASE + and STATISTICA. **Results:** Although a pre-amplification recommended for quantification from small starting RNA amounts was used, only 6 lncRNAs were detected in all plasma samples ($Cq < 35$). 11 lncRNAs were not detected in any samples. No significant difference was observed in expression of plasma lncRNAs between the BC patients and healthy donors despite the fact that our panel contained the lncRNAs whose expressions were previously reported as significantly different at the level of cancer and control tissues. **Conclusion:** Detection of lncRNAs in plasma is due to its low levels quite difficult as compared with tissues. Our findings suggest that plasma lncRNAs are not suitable for use as non-invasive diagnostic tools in BC patients.

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E-P17.04

Natural epigenomic variations manifesting as long contiguous stretches of homozygosity spanning the imprinted genes

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Introduction Natural epigenomic variations in humans are poorly understood. For instance, long contiguous stretches of homozygosity (LCSH) are consistently observed to be abundant in a human genome, but the role of these epigenomic variations in behavioral variability of the genome remains to be established. Recently, it has been shown that LCSH spanning loci of imprinted diseases (i.e. Angelman and Prader-Willi syndromes) are associated with intellectual disability, autism and epilepsy (Iourov et al., 2015). Here, we have attempted to evaluate spectrum of epigenomic variations (LCSH) involving imprinted genes, which are not associated with a specific disease. Methods Using SNP-array molecular karyotyping (Affymetrix), we evaluated genomes of 350 children with intellectual disability and ASD. An original bioinformatics technique (Iourov et al., 2014) and a gene imprint database were applied to address LCSH pathogenicity involving imprinted genes. Results LCSH involving imprinted genes was found in 263 (75%) individuals. LCSH encompassed following imprinted genes: *PSIMCT-1* (33.4%), *LIN28B* (8%), *RBI* (7%), *ZC3H12C* (6.9%), *DNMT1* (5.4%), *MAGI2* (5.1%), *NPAP1* (3.7%). Proportions of individuals exhibiting these LCSH are given in parentheses. We have not found these epigenomic variations to possess phenotypic effects. Conclusions This study evidences for an additional type of

interindividual (epi)genetic variations, which are likely to be benign in cases of the aforementioned genes in contrast to imprinted genes located at 7q21.3, 7q31.2, 11p15.5, 15p11.2 (according to Iourov et al., 2015). These natural epigenomic variations should be taken into account in future diagnostic and basic genomic/epigenomic studies. Supported by the Russian Science Foundation (project #14-15-00411).

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E-P17.05

Investigation of two CpG islands of Monoamine Oxidase A (MAOA) gene promoter among opium addicted males undergoing methadone treatment

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It has been demonstrated that methylation of MAOA gene promoter is associated with alcohol and nicotine dependence in women, not in men yet. Antisocial personality disorder (ASPD) and substance use disorders (SUD) are two types of disorders which could highly be influenced by methylation induced changes in MAOA function. The aim of the current study was to investigate the effect of opioid addiction on methylation of MAOA gene promoter in males. DNA was extracted from the whole blood of all samples (29 heroin-addicted individuals undergoing methadone treatment and 28 healthy people) according to the extraction protocol and then were treated with bisulfite kits. The investigated region including two CpG islands in promoter region of MAOA gene contained 35 CpG dinucleotides which investigated by Sanger sequencing method. The statistical analyses of total methylation percentage between addicted individuals undergoing MMT and healthy people were indicated no significant differences between two groups among 35 CpG dinucleotide sites. In conclusion, there is no association between 35 studied CpG sites of two CpG island in the promoter region of MAOA gene with opium addiction among males.

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E-P17.06**Circulating microRNA signature as novel biomarker for osteoarthritis development**

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Osteoarthritis (OA) is a heterogeneous joint disease that leads to joint malfunction and eventually disability. As there are no therapies to halt disease progression, we aimed to identify a circulating miRNA signature for the discrimination between OA patients and controls. Serum samples were collected from 12 patients with primary OA and 12 healthy individuals. Microarray screening was performed using Agilent Human miRNA Microarray, followed by validation with real-time PCR. Multi-parameter analyses and ROC curves were conducted with MATLAB simulation environment. MiRNAs that were found deregulated between OA and healthy cartilage were further analyzed by Webgestalt, TargetScan and DIANA bioinformatics web-tools. We identified 279 differentially expressed miRNAs in OA patients' serum compared to controls ($p < 0.05$ και FDR < 0.05). 205 miRNAs (73.5%) were upregulated and 74 (26.5%) downregulated. 6 miRNAs were validated by real-time PCR and confirmed the microarray data. Among them, miR-33-3p and miR-4284 had the highest down-regulated values and AUC > 0.8 in ROC analysis. Bioinformatics analysis revealed that the deregulated miRNAs were involved in the TGFb, adherens junction pathway and biosynthesis of unsaturated fatty acids, as well as proteoglycans, glycosaminoglycan, AMPK and mTOR signaling pathways among others. Furthermore, it appeared miRNA enrichment showed participation in inflammation biological functions. MiRNA arrays along with bioinformatics analyses could be proven a useful tool for the identification of prognostic and therapeutic targets in osteoarthritis. MiR-

33-3p and miR-4284 could be proposed as novel biomarkers for early osteoarthritis development. *These authors contributed equally. This project is supported by fellowships of excellence- IKY/ SIEMENS.

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E-P17.07**BRAF mutation and hypermethylation of RASSF1A, GSTP and RARbeta2 in prostate cancer patients**

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Introduction: Previous studies demonstrated that BRAF mutation in thyroid cancer is mutually exclusive with RASSF1A promoter hypermethylation but often occurs together with methylation of RARbeta2. As RASSF1A, RARbeta2 and GSTP hypermethylation is common in prostate cancer we were aimed to check their interrelation with BRAFV600E mutation in this type of tumor. Materials and Methods: DNA from 18 urine samples of cancer patients and 6 non-cancer individuals was isolated by phenol-chlorophorm method. All samples were analyzed for the presence of normal BRAF alleles. After bisulfite treatment MSP Real-time PCR was performed to detect hypermethylation of RASSF1A, GSTP and RARbeta2 promoters. Results: Normal BRAF allele was found in all 24 samples, BRAFV600E mutation was detected in one tumor and one normal sample. Methylation of RASSF1A was present in 17/18 tumor samples, GSTP and RARbeta2 promoters were hypermethylated in all samples from cancer patients. One sample from non-cancer individuals had all three genes hypermethylated. Other normal samples showed hypermethylation of one or 2 genes of set. In normal sample with BRAF mutation we found hypermethylation of RASSF1A and RARbeta2. Conclusions: We didn't find any correspondence between BRAF mutation and hypermethylation of studied genes. Hypermethylation of them in non-cancer individuals might be caused by the senior age of volunteers, thus we suggest that this set is not specific enough and need to be completed.

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E-P18 Genetic epidemiology/Population genetics/Statistical methodology and evolutionary genetics

E-P18.01

Hemoglobin H disease in Khuzestani patients of Iran

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Introduction: We studied the α -globin gene genotypes, hematologic values, and transfusion dependency of patients with hemoglobin H (HbH) disease in Khuzestan Province, Southwest of Iran. **Methods:** The alpha globin gene mutation was detected by using Gap-PCR and direct DNA sequencing. **Results:** We identified 120 patients with hemoglobin H disease. Of these patients, 35 (29.16%) had deletional form of HbH disease, and 85 (70.83%) had different form of nondeletional HbH disease. The most frequently observed HbH genotypes were --Med/ $-\alpha 3.7$ in 33 patients (27.5%), α CD19(-G) α/α CD19(-G) α in 25 cases (20.83%), α polyA4 α/α polyA4 α in 15 (12.5%), and α polyA6 α/α polyA6 α in 13 (10.83%) respectively. The probability of receiving at least one transfusion blood in deletional form was observed in 3 of 35(8.57%) patients which just seen in 3 of 33(9%) patients with --Med/ $-\alpha 3.7$ genotype. This form was also observed in 8 of 85 (9.4%) patients in nondeletional HbH diseases. **Conclusions:** The diagnosis of Hb H disease at the molecular level is important with respect to genetic counseling and the identification of families at risk for having pregnancies affected with Hb H disease. Regarding the need for blood transfusion in deletional and non deletional HbH disease, the most of HbH cases was not associated with an increased rate of severe anemia and was managed without blood transfusion. Therefore, we can recommend that Med deletion in compound with alpha globin point mutations and Constant spring in homozygous form can be consider as thalassemia syndrome need for blood transfusion.

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E-P18.02

Reconstructing the human population history of the

Indian subcontinent using ancient population genomics

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Normal 0 false false false EN-US X-NONE X-NONE MicrosoftInternetExplorer4 /* Style Definitions */ table {mso-style-name: "Table Normal"; mso-style-rowband-size:0; mso-style-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-qformat:yes; mso-style-parent:""; mso-padding-alt:0in 5.4 pt 0in 5.4 pt; mso-para-margin:0in; mso-para-margin-bottom:.0001 pt; mso-pagination:widow-orphan; font-size:11.0 pt; font-family: "Calibri", "sans-serif"; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-fareast-font-family: "Times New Roman"; mso-fareast-theme-font:minor-fareast; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family: "Times New Roman"; mso-bidi-theme-font:minor-bidi;} The more than 1.3 billion people who live in Indian subcontinent correspond to several large ethnic groups who are highly diverse and complex. Importantly, India's genetic past remains a subject a great debate due to numerous hypotheses surrounding population origins and migrations within and from outside India. In order to reconstruct and explain the patterns of genetic diversity evident in modern humans, an understanding of both past and present population dynamics is crucial. Several studies have shown that genetic data from ancient individuals are indispensable when reconstructing past population histories. We for the first time use the ancient genomics approach in South Asia to reconstruct the complex human population history of Indian Sub continent. We are exploring the recent technological advancement to directly test these hypotheses using ancient and modern human DNA in India. We have collected several ancient skeletal remains from different time scale of human civilization ranging from early Mesolithic, Neolithic, Harappan (Indus Valley civilization) and Megalithic culture. With the whole/partial genome NGS data, we are reconstructing the prehistoric peopling and migration of modern human in the Indian subcontinent. We are also testing the pervasive founder events and gradient of recessive genes accumulation by comparing the ancient genome with the modern human population of India.

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E-P18.03**Genomic analysis of ethnic regions in Armenia**

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Introduction: ArmGenia is a joint United States/Armenia initiative with the primary goal of determining genetic variants specific to the Armenian population. Given the unique historical features of this genetically isolated population, going back thousands of years, this initiative will provide scientific and health benefits for both the Armenian and international communities. **Materials and Methods:** Armenian individuals ($n = 25$) traced to Bayazet, Erzrum, Artsakh and Kharpertzi were recruited. Leukocyte DNA was subjected to whole-exome sequencing. Principal Component Analysis (PCA) was performed on 15,000 common, single-nucleotide polymorphisms from the Armenian and from 200 companion exomes representing over 10 worldwide populations. In addition, the Armenian individuals' carrier status for selected autosomal recessive disorders was determined. **Results:** PCA analysis showed that Armenian genomes were genetically most related to American/European Caucasians and Ashkenazi-Jewish individuals, while appearing distant from Mexican/Hispanic, Indian, and African populations. Based on preliminary data, individuals from Bayazet were most distant genetically from European/American Caucasians. Known mutations in the *MEFV* gene causing familial Mediterranean fever were detected at frequencies expected (17.5%), but surprisingly, mutations for recessive disorders more typical of other ethnic groups, including Gauche disease, Fanconi anemia type C, and phenylketonuria, were identified. No deleterious variants were found in the major genes associated with familial breast/ovarian and colon cancer. **Conclusions:** These data confirm European origin of the Armenian populations in these regions, with attendant carrier status for some European recessive disorders. We continue to analyze these genomes for disease-causing and modifier variants for other disorders of public health importance to Armenia.

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E-P18.04**Epigenetic effect of butylated hydroxytoluene (BHT) on BDNF gene methylation, learning and memory in male wistar rat**

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BDNF gene has important roles in the development of the nervous system and neuronal plasticity-related processes such as memory and learning. A lot of studies have demonstrated that BHT can play as an epigenetic factor in human and other species. The aim of current study is to investigate the way it can affect the methylation status of BDNF gene, learning and memory. In current study, 21 male Wistar rats weighting 190–210 gr were used. Animals were divided in to tree groups: 1-control group (received same oil with the same volume as experimental group), 2-experimental group (received BHT 60 mg/kg/day), and 3-intact group (without injection). BHT was administrated by IP injection for 14 days. Learning and memory were assessed by passive avoidance shuttle-box. The methylation status of 12 CpG sites located in one CpG island of BDNF promoter region were assessed by sequencing after treatment of extracted DNA with sodium bisulfite. Data were analyzed by one way anova, tucky's post-hoc test, flisher'exact test. The learning ability and memory did not show statistically significant difference between three groups in first day($p = 0.139$). However, they represented statistically significant difference between three groups in second day ($p = 0.0001$). The frequency rate of methylation did not statistically demonstrate significant difference between groups (intact vs BHT, control vs BHT and control vs intact). We concluded that BHT can affect the learning ability and memory of male Wistar rats. However, it not able to create methylation in the BDNF gene.

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E-P18.07**Monitoring of congenital anomalies among the population lives near nuclear power plants**

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Introduction: The knowledge of the frequency of congenital anomalies (CA) in the population requires for organization of their prevention. Purpose of the study is determining the incidence of CA among the population living near Nuclear Power Plants (NPP) in Russia. **Material and Methods:** Register CA operates since 2000 in according to recommendations EUROCAT and Clearinghouse (ICBDMS). Information about all the identified CA among the population living in urban areas near the nuclear power plant arrives into the register. Genetic counseling of families is carried out, if necessary. Labour conditions of parents of children with CA are also taken into account. **Results:** We found no significant differences in frequency among the VA the population living in towns near Russian NPPs and in the regions of their location during the observation period from 2000 to 2004. The average frequency of CA in 36 regions of Russia is 6,14%, it's significantly higher than the average frequency among the living in the towns near the nuclear power plant population (4,95%). We have shown no association of the child's CA and labor conditions of parents. **Conclusions:** It was shown the possibility of using the CA frequencies data for prevention. At the same time, the volume of accumulated information is too small to produce reliable estimates for conclusive findings now. It is necessary to continue the research, expanding its scope and compared with those obtained in other countries with the results.

P.V. Izhevskiy: None. **V.L. Izhevskaya:** None.

E-P18.08**Prevalence of consanguineous marriages and associated factors in Morocco**

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Introduction: Consanguineous marriage has received a great deal of attention as a potential risk factor for many adverse health effects. The present study aims to determine the prevalence of consanguineous marriages and associated factors in Rabat, Morocco's capital. **Materials and Methods:** A prospective study was conducted within a randomly

selected sample (810 couples). All information was based on structured face-to-face interviews. **Results:** The results indicate that 21% (95% CI: 12.3–28%) of marriages are consanguineous, among which 70% are between first cousins. According to these results, educational level, place of residence (urban or rural), childhood residence and the age at first marriage have an influence on the choice of first spouse for women. Multivariate analysis of data shows that the probability of marrying a relative is significantly higher among women who spent childhood in the countryside: they have eighteen times more likely than their urban counterparts. Moreover, according to the results, women who marry at a young age are more likely to accept this type of alliance, that is, five times more than the women who marry later. However, these two variables have no significant effect among men. **Conclusions:** Specific health education and genetic counseling should be followed in line with WHO recommendations to minimize the negative effects of consanguinity on child health.

H. Hami: None. **A. Mokhtari:** None. **A. Soulaymani:** None.

E-P18.09**Marriage patterns and consanguinity in North West Morocco**

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Introduction: More than one billion people worldwide live in regions where 20%–50% of marriages are consanguineous. The aim of this study is to determine the frequency and types of kinship in the region of Tanger-Tétouan in North West Morocco. **Materials and Methods:** A prospective study was conducted among a randomly selected sample of 213 university students in Tanger in 2014. All students were interviewed using a structured questionnaire. **Results:** The frequency of consanguineous marriages among parents of students was 45.3% (95% CI: 48.6–61.3%). The study revealed a statistically significant difference in the rate of consanguineous marriages between the current (parents') generation and the previous generations ($p < 0.001$). The most common types of consanguineous marriages among the current generation were between first cousins (77.4%), in particular patrilateral parallel cousins, followed by first cousins once removed (8.3%) and second cousins (2.2%), while 12.1% of marriages were between distant relatives. **Conclusions:** Consanguineous marriage plays an important role in expression of deleterious recessive genes. Public awareness of genetic

risks associated with consanguineous marriage and the importance of premarital genetic counseling are indispensable.

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E-P18.10

CYP2D6 *3 and *4 alleles frequencies among Volga Tatars

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INTRODUCTION. CYP2D6 is a member of the cytochrome P450 gene superfamily which contributes to the metabolism of up to 25% of clinically used drugs. The interindividual variations of treatment response and toxicity of metabolized drugs are influenced by the polymorphism of the gene and strongly depends on ethnicity. So, the aim of this study was to determine the frequencies of “slow” allele variants CYP2D6 *3 and CYP2D6 *4 in ethnic group of Tatars. **METHODS:** Genotyping of CYP2D6 *3 and *4 alleles (rs35742686 and rs3892097 respectively) was performed on DNA samples extracted from venous blood from 583 unrelated persons of Tatar ethnicity from Republic of Tatarstan (Russia) using real-time PCR with TaqMan probes. The statistical analysis was performed using R program package. **RESULTS:** In Tatars one of the most important “slow” allele variants CYP2D6 *3 had frequency of 0.6% which is between the Europeans (2%) and Mongoloids (0.2%) whereas the frequency of CYP2D6 *4 was 11.2% which is much closer to the Asian populations frequency (11% in Mongoloids vs 19% in Europeans). However, 76.7% were identified as homozygous extensive metabolisers (EM) with no mutant alleles, almost 23% were carrying one mutant allele (heterozygous EM) and only 2 persons (0.34%) were classified as poor metabolizers that were carrying both mutant alleles. **CONCLUSION:** Since allele variants of CYP2D6 gene can play essential role in interindividual and in interethnic differences in the metabolism of many therapeutic agents, the obtained results could be used in the prognosis of pharmacotherapy efficacy in populations of Tatars.

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E-P18.11

Molecular epidemiology of common hereditary disorders in Karachay-Cherkess Republic

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Introduction: This research is a first medical genetic examination of 10 populations of the Karachay-Cherkess Republic for common hereditary autosomal recessive disorders: cystic fibrosis (CF), sensorineural hearing loss (SNHL), and phenylketonuria (PKU).

Materials and Methods: Total amount of examined population is 317 956 individuals, including 43.1% Karachays, 24.3% Russians, 14.7% Cherkess, 9.4% Abazins, 3.9% Nogais, and 4.5% of others. In total, 177 patients underwent DNA diagnosis for three the most common autosomal recessive hereditary diseases: 16 patients for CF, 111 for CNHL, and 50 for PKU. Molecular diagnosis was performed with PCR, MLPA, and Sanger sequencing.

Results: CF was diagnosed in 21 patients from 20 unrelated families. Disease prevalence appeared to be 1:2647 according to newborn screening results. Molecular diagnosis of 15 Karachay patients revealed high prevalence of W1282X mutation (93.3% of affected *CFTR* alleles) and absence of F508del mutation. Haplotypes analysis showed W1282X mutation has different origin in Karachay and East European patients. Single Russian patient have F508del/2184insA genotype. Isolated SNHL was diagnosed in 156 families (205 patients) resulting in incidence 1:1551. *GJB2*: c.35delG mutation prevalence was determined in 111 patients from 102 families of different ethnic background. PKU prevalence was determined as 1:1638 live births. Common mutations in the Karachay population are R261X (86.25% of affected *PAH* alleles), R413P, P211T, F331S.

Conclusions: the most common autosomal recessive hereditary diseases prevalence was estimated in populations of the Karachay-Cherkess Republic and showed national peculiarities in mutation spectrum and frequencies. The research was partially supported by grants RFFR 17-04-00288, 15-04-01859; RSF 17-15-01051.

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E-P18.12

CFTR mutations in Chechen CF patients

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Cystic fibrosis (CF; OMIM #219700) is a common autosomal recessive disease. Detection of the mutation spectrum in specific population is one of the task of genetic epidemiology. Chechens are a Caucasian ethnic group of the Nakh peoples originating in the North Caucasus region. The Chechen people are mainly inhabitants of Chechnya (Russian Federation). Molecular genetic analysis for 32 *CFTR* mutations (shared up to 80–85% of mutant alleles in population from Russian Federation) was performed in 34 Chechen CF patients from 33 unrelated families. A high proportion of 1677delTA and E92K mutations was revealed (45 and 10 out of 68 mutant alleles, 66.2% and 14.7%, correspondingly): 15 patients - homozygous for 1677delTA, 9 - compound heterozygous for 1677delTA and E92K. Each of A96E, R334W and W1282X mutations were found on one mutant chromosome. 14.7% of mutant *CFTR* alleles were still unidentified in Chechen CF patients. Both 1677delTA and E92K mutations were associated with the haplotype 22-7-16-13 of four linked DNA markers, IVS1CA-IVS6aGATT-IVS8CA-IVS17bCA. Up today the highest frequency of 1677delTA mutation was noted in Georgian CF patients (about 60%). 1677delTA mutation was determined in other ethnic groups of the North Caucasus such as Karachai, Cherkess, Abaza, Ingush. A relatively high frequency of E92K mutation associated with the same 22-7-16-13 haplotype was found in populations that are historically associated with the settlement of Turkic peoples in the Volga-Ural region (Chuvash, Tatars, Bashkirs), but not in North Caucasus populations (Karachai, Nogai). The research was partially supported by grants RFFR 17-04-00288, 15-04001859, RSF 17-15-01051.

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E-P18.13

allelic frequencies of single nucleotide polymorphisms in TPMT and NAT2 genes in the Bahraini population

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Introduction The thiopurine S-methyltransferase (TPMT) and N-acetyltransferase 2 (NAT2) enzymes play a crucial role in the metabolism of xenobiotics. Mutant alleles of these enzymes are involved in adverse drug reactions.. Population studies have demonstrated significant ethnic differences in the distribution of TPMT and NAT2 variant alleles among various ethnic groups. The aims of this study were to determine the frequencies of allelic variants in the *TPMT*, and *NAT2* genes in the Bahraini population and compare them with the frequencies in other ethnic groups.

Materials and Methods DNAs were isolated from peripheral blood leucocytes of 265 healthy Bahraini volunteers. TPMT*2 was detected using an allele-specific polymerase chain reaction (PCR) assay. PCR-RFLP assay was applied for the determination of TPMT*3 A (*3B). TPMT*3 C and NAT2 variants (*5,*6 and *7) were detected using an allele-specific real-time PCR assay.

Results Genotyping of TPMT revealed frequencies of 0.949, 0.008, 0.008 and 0.036 for TPMT *1, *2, *3B, and *3 C, respectively. No TPMT*3 A was detected in the analyzed samples. The frequencies of specific NAT2 alleles were 0.150, 0.405, 0.397 and 0.047 for *4 (wild-type), *5 (T341C), *6 (G590A) and *7 (G857A), respectively. The percentages of the rapid, intermediate and slow acetylator genotypes was 4.04%, 31.39% and 64.57%, respectively.

Conclusions This study provides the first data on the frequency of common TPMT and NAT2 variants in the Bahraini population. The study showed the observed genotypic similarities and differences of Bahrainis with other Asian populations and Caucasians. Future studies will investigate for new unique polymorphisms among Bahrainis.

A.A.S. Deifalla: None. **M.A.A. Ali:** None.

E-P18.14**Detecting the recent changes of effective population size in the Lithuanian population****A. Urnikyte, A. Molyte, V. Kučinskas**

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Introduction: There are two important parameters to be estimated in natural populations: the effective population size (N_e) and census size (N). Knowing the ratio of N_e/N is useful for estimating effective population size from census data and for examining how different ecological factors influence N_e . The main interest of this study was to reconstruct the recent N_e by using IBD segments and relate this to census size in the Lithuanian population. **Methods:** We used Illumina 770 K HumanOmniExpress-12v1.0 array data of 295 unrelated individuals of the Lithuanian population. IBDseq v. r1206 and IBDNe v. 04Sep15. e78 software packages were used to detect IBD segments and to estimate N_e respectively. **Results:** We estimated the recent N_e for 50 generations in the Lithuanian population. The evaluated N_e/N ratio was 0.125 (95% CI [0.077; 0.271] for $g = 2$ (corresponds to 1941), 0.114 (95% CI [0.065; 0.280] for $g = 1$ (corresponds to 1966), and 0.124 (95% CI [0.065; 0.341] for $g = 0$ (corresponds to 1991). The estimates of N_e were approximately one-tenth of the Lithuanian census population size. **Conclusions:** We conclude that natural levels of fluctuations such as variance in size, reproduction, sex ratio, and the degree to which generations overlap probably influenced the small values of N_e/N in the Lithuanian population. Considering our results however, we think that the true N_e is contained within the bootstrap confidence interval. This work supported by the LITGEN project (VP1-3.1-ŠMM-07-K-01-013), funded by the European Social Fund under the Global Grant Measure.

A. Urnikyte: None. **A. Molyte:** None. **V. Kučinskas:** None.

E-P18.15**Exome sequencing of an Arabian set reveals diverse novel variants****A. M. Alkhateeb**

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Arab genome is poorly represented in human genome databases such as the 1000 genome project and HapMap. Arabs represent a unique genetic structure due to widespread endogamous and consanguineous marriage and to admixture over the centuries with migrating subcultures of African, Asian and European ethnicities. With the objective of getting better understanding of the genetic structure of

Arabian genome and examine its diversity we analyzed the genome of six apparently-normal unrelated males of Arabian decent. Whole exome sequencing is done on all individuals with mean target average for all samples 108 \times . Sequences were analyzed using Ingenuity Variant Analysis (IVA) tool. Excluding variants presented in dbSNP and variants with at least 0.001 allele frequency in either ExAC, NHLPI ESP, 1000 genome project, or Allele frequency Community in Ingenuity database we got 2377 variants from our samples. Overall frameshift mutations were 306. Eight Pathogenic mutations and 13 likely pathogenic mutations were identified. In this work we discuss the uniqueness of our local population genome and the novelty of mutations present

A.M. Alkhateeb: None.

E-P18.16**Distribution of Factor V Leiden, Prothrombin G20210A and MTHFR C677T mutations in Georgian population**

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Introduction: Inherited Thrombophilia is a blood coagulation disorder that increases the risk for venous thromboembolism (VTE), arterial thrombosis and pregnancy complications. Family-based studies have indicated that more than 60% of variations in susceptibility to thrombosis are attributable to inherited components. Although more than 99,5% of genetic material is conserved among different ethnicities, the frequencies of variants and common genetic factors for thrombosis vary widely. There are few studies that directly compare the variation in distribution Factor V Leiden, Prothrombin G20210A and MTHFR C677T gene mutations according to ethnicity. According to this fact, that distribution of above mentioned mutations in Georgian population hasn't been studied before, the aim of our study was to detect and compare the rates of distribution of these mutations among Georgian population and different ethnic groups. **Materials and Methods:** 1340 unrelated Georgians were genotyped by PCR analyses for last seven years. **Results:** distribution of studied mutations in Georgian general population is high and resembles upper data of Caucasians. Results are presented in a table:

Conclusions: This is the first study in our population and shows that inherited thrombophilia has significant impact

Distribution of mutations in different populations				
General Populations	FVL-1691A Pr-20210A MTHFR-677T			
Georgian	4.57%	3.72%	46.1%	
Caucasian	North Europe	1.1%–7.3%	0.6%–2.4%	32.7%
	Central Europe	0.6%–5.1%	–	–
	South Europe	0.9%–4.0%	1.1%–4.0%	62.5%–79.2%
African		1.0%–10.2%	0.0%–3.7%	11.9%
Hispanic		0.4%–1.4%	1.3%–2.0%	47.9%
Asian	East Asia	0.0%	0.0%	1.1%–16.7%
	West Asia	2.1%–3.8%	0.0%–1.8%	47.7%

on development of blood coagulation disorders in Georgian population. Understanding importance of these results will help clinicians and healthcare professionals to manage thrombosis and develop prevention program. The study has been funded by the grant DO/166/7-140/14 of SRNSF of Georgia.

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E-P18.17

Age-at-onset in descendants of late-onset cases of TTRVal30Met related familial amyloid polyneuropathy (FAP)

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Familial amyloid polyneuropathy (FAP) is an autosomal dominant amyloidosis, due to a point mutation in *TTR*. We confirmed anticipation of age-at-onset (AO) and found a parent-of-origin effect. Our aim now was to explore families with late AO (≥ 50). From the data registry at Unidade Corino de Andrade, we analysed the descendants of late-onset cases. For statistical analysis, SPSS v.23 was used. Among 178 offspring of those initial late-AO cases, 105 (59%) had early AO (< 40) (59% males, 41% females). Moreover, 65% inherited the disease from their mother, while 35% from the father ($p < 0.05$). In contrast, 73 (41%) had AO ≥ 40 (47% males, 53% females), with no parent-of-origin effect. In the 3rd generation, only two individuals

(2.9%) had AO ≥ 40 , while 30 were still asymptomatic (44.1%). The remaining 36 (52.9%) presented early AO. Within the asymptomatic group, we found a mean higher age-at-observation when the transmitting parent had AO ≥ 40 . None of the offspring in the 2nd or 3rd generation had an onset later than their 1st generation. We found anticipation in most of the siblings, showing that this is not an isolated event. When anticipation of AO was seen, the reverse could not be found in subsequent generations. We confirmed a parent-of-origin effect, but associated only with the transition from late to early AO. Due to the different clinical presentation of FAP in later-onset patients and the abrupt decrease in AO often seen in just one generation, it is crucial to explore the mechanisms that may lead to this phenomenon.

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E-P18.18**Association study of polymorphic variations with haemoglobin A2 levels in beta-thalassemia carriers**

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Introduction: Elevated HbA2 levels provide diagnostic evidence for beta-thalassemia trait. This study aims to examine the genetic basis of HbA2 variability in beta-thalassemia carriers using SNP variations previously reported associated with HbF. **Material and Methods:** Sixty one β-thalassemia carriers of Portuguese descent (34 females and 27 males) aged 2–77 years (mean 32.7), with HbA2 levels ranging 3.4–6.8% (mean 4.78), were selected. HbA2 and HbF levels were determined by HPLC. Twelve SNPs in loci *BCL11A*, *DCHS2*, *HMIP*, *HBG2*, *RNF113B*, *GLP2R* and *KLF1* were genotyped using TaqMan assays or by PCR-RFLP. Informed consent was provided by all the participants. Associations of SNPs with HbA2 levels were assessed by linear regression under a dominant genetic model using PLINK. **Results:** Linear regression showed nominal significant association between the rs61746132 minor A-allele on *DCSH2* gene and HbA2 levels after adjustment for age and sex ($P = 0.039$), remaining significant after adjustment for HbF ($P = 0.039$). A nominal significant association was found between the rs3817621 minor C-allele on *KLF1* gene and HbA2, unadjusted ($P = 0.019$) and adjusted for age and sex ($P = 0.038$), however the significance was lost when adjusting for HbF. No nominal significant associations were observed for the remaining analysed SNPs. **Conclusions:** Our results suggest a relationship between HbA2 levels and rs61746132 located at *DCSH2* gene which is overexpressed in bone marrow stromal cells. The *KLF1* gene promoter common variation rs3817621 was found significantly associated with HbA2 levels which appear to be mediated by the effects of this locus on HbF expression.

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E-P18.20**Molecular-epidemiologic and epigenetic study of****HPV infection in cervical samples of Bulgarian patients**

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Introduction: Cervical cancer is tightly connected to HPV infection, but it is not yet elucidated why some of infected cases progress to cancer, but others not. Our aim was to study the incidence of HPV types in samples of Bulgarian patients from different PAP groups and to study the role of DNA methylation in the progression of HPV infected cervical samples. **Materials and methods:** We genotyped 19 high/medium risk HPV types in 69 cervical samples by allele-specific hybridization. Then we measured hypermethylated DNA fraction in promoters of 22 genes, connected to cellular stress and oncogenesis by real-time PCR arrays in two HPV positive groups: PAP I/II and PAP III/IV. **Results:** The most prevalent were HPV 16 (31.9%), followed by HPV 66 (29%) and HPV 56 (14.5%). There was statistically significant difference between PAP groups - 75% of PAP III/IV samples were HPV positive versus 43% of PAP I/II samples. In PAP III/IV we found an increase in hypermethylation of more than 1.5 times for genes Rara, Gadd45G, BNIP3, SCARA3, Mlh1, Xpc and CDKN1a. **Conclusions:** We suggest an association between HPV cervical progression and hypermethylation (inactivation) of important tumor-suppressor genes and genes, connected to oxidative stress protection.

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E-P18.21**Detailed study of the genetic structure of the Volga-Ural region populations**

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73 mtDNA haplogroups were identified in populations of the Volga-Ural region. The largest contribution to the gene pool of mitochondrial haplogroups in the region was made by the lines H (24.9%), U (20.7%) and T (11.5%) - together they account for about half of all mtDNA haplogroups in the Volga-Ural region. In the populations of the Volga-Ural region 33 mitochondrial genomes were completely sequenced, all belonging to the paragroup H * (samples not related to the H1-H14 lines). Three populations were identified with haplogroup H55: Mari (8.6%), Arkhangelsk Bashkirs (6.8%) and Chuvash (2.9%). In the Udmurt population a new haplogroup H99 was discovered with a frequency of 4.6%. Most of the Y-chromosome gene pool in the studied populations falls into three haplogroups: R-M269, R-M198 and N-M231, with frequency ranging from 49% to 100% in different populations. Based on the research on the new markers (Pamjav et al, 2012;.. Underhill et al, 2014), we conducted a study of the individual branches of haplogroup R1a-M198. It has shown that of the European subhaplogroup (R1a-M458 and R1a-M558) R1a-M558 is more common in the populations of the Volga-Ural region, it was identified by us in almost all populations of the Volga-Ural region, with the exception of the Bashkirs. Mostly Asian haplogroup R1a-Z93 in the Volga-Ural region is represented by three lines: R1a-Z93 (xZ95), R1a-Z95 (xZ2125), R1a-Z2125. Our results show that R1a-Z93 (xZ95) and R1a-Z95 (xZ2125) are present at low frequencies in populations of the region, while R1a-Z2125 is dominant in subpopulations of Bashkir.

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E-P18.22**Reconstruction of the early population history of****Africans in the Americas through St. Helena Island (South Atlantic) and New York City**

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Improved sequencing technologies allow for ancient DNA research of historic remains of underrepresented groups, which can provide riveting insights into their biological history. The combination of molecular genomic analyses and morphometric anthropometric assessments, within bioarchaeological and historical contexts, give a more accurate perspective on the significant events that occurred in the past. The investigation focuses on the status of early to mid-19th century Africans on their way to enslavement in the Americas and the status of late to 18th through mid-19th century enslaved Africans and African Americans in New Amsterdam/New York City. This investigation reconstructs the early population history of Africans in the Americas using these two important source populations. There is an examination of the range of demographic and morphometric diversity discovered among the liberated Africans buried at St. Helena. A goal is to determine if there is any similarity with that observed among individuals found in the New York African Burial Ground. It is hypothesized that skeletal remains on St. Helena show evidence of disease and trauma similar to the skeletal remains found on the New York African Burial Ground. Furthermore, the suggestion is that the range of ancestral genomic variability found in the South Atlantic African remains is similar to the range of genomic diversity observed in the New York African Burial Ground. Together, these sites provide a continuum of insights into the early population history of these African peoples.

G. Johnson: None. **F. Jackson:** None.

E-P18.24**Polymorphism of nine nuclear genome DNA loci in Karachays in comparison with other autochthonous populations**

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The Karachay-Cherkess Republic, Russian Federation, is a region of compact residence of four different ethnic groups: the Karachays, Cherkess, Abazins, Nogais. The aim was to carry out the population genetic survey of one of the indigenous population of the Karachay-Cherkessia Republic, Karachays, and compare it with other ethnic groups, Abazins, and Nogais. The Karachays are a Turkic-speaking people of the North Caucasus. Methods: DNA samples from healthy unrelated individuals (383 individuals) were examined for nine polymorphic DNA loci of nuclear genome: diallelic markers CCR5 (del32), ACE (del/ins), D7S23 (KM19), NOS3 (VNTR), and polyallelic loci THOI (STR), FABP2 (STR), CFTR (IVS6aGATT), PAH (VNTR), DAT1 (VNTR) by PCR. Results: Allele and genotype frequency distributions were obtained for Karachays. Analysis of alleles frequency of autosomal DNA markers revealed genetic differentiation between populations Karachays and other indigenous populations of the Republic. The highest level of genetic diversity in a diallelic system was estimated for the locus ID/ACE, $H_{obs} = 0.4960$, in a polyallelic system - for the locus THOI (STR), $H_{obs} = 0.7804$. The index of mean heterozygosity was 0.4565. The index of mean intrapopulational differentiation (F_{ST}) was 0.0101. The highest level of intra-populational differentiation was revealed at locus VNTR/eNOS ($F_{ST} = 0.0216$), the lowest one - at the locus KM19 ($F_{ST} = 0.0027$). The rate of subethnic differentiation in Karachays appear to be the highest among the studied populations: Nogais and Abazins. The research was partially supported by grants RFFR 17-04-00288, 15-04-01859; RSF 17-15-01051.

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E-P18.25

Investigation of relative leukocyte telomere length in Iranian healthy individuals

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Telomeres are repetitive sequences at the end of eukaryotic chromosomes which are responsible for genomic integrity. It has been proven that telomere length is a good

marker of biological age. Thus relative leukocyte telomere length (RTL) in Iranian healthy individuals was measured by monochrome singleplex qPCR. To calculate T/S ratios (telomere amplification/ single copy gene amplification (36B4)), the samples ran in duplicate in the same well position, for three times. Telomere and 36B4 gene amplification were done in different plates. All the samples quantified against a 5 point standard curve using a mixture of 5 genomic DNA with different chronological ages as a reference DNA. K562 genomic DNA was included in each plate as a quality control and to avoid more than 5% coefficient of variation between different plates. So far, 116 individuals including, 53 women and 63 men (29–85 years) from two different cities, were selected. They all had same ethnicity (Persian), and lived at least ten years in their own city. They were not related. They were nonsmokers and had no sign of chronic infections or cancer. Complete clinical and cardiovascular examination were done for each individual. By classifying the samples in 10-year age groups, we found a reverse correlation between chronological age and length of telomeres ($R = -0.996$). Due to restrict criteria for sample selection, good linear regression with $R^2 = 0.989$ and $P\text{-value} = 0.0005$ has been observed. Further analysis in a bigger sample size and for different sex groups is ongoing.

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E-P18.26

An evaluation of *de novo* mutation content in the Lithuanian exome

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De novo mutations (DNMs) are the ultimate source of genetic variation and one of the driving forces of evolution. Early studies of mutation rates in humans focused on relatively small and specific loci or on the *de novo* incidence of disease. Family-based whole-genome sequencing has begun to identify DNMs that provide more direct measures of mutation rates. Therefore in our study we have focused on the DNM content and intensity of Lithuanian whole exome's data. Sequencing of 48 trios exomes was performed using SOLiD 5500 system. Sequencing data was generated by Lifescope. DNMs called by two alternatives programs, VarScan and VarSeq. Called DNMs were filtered by applying the genotype quality and number of reads filters. All called and filtered possible DNMs were manually reviewed by the IGV and annotated using ANNOVAR or

specific VarSeq annotating tool. R package was used to estimate rate of DNM. We estimated 189 (VarScan) and 121 (VarSeq) DNMs respectively. In our calculation DNM rate is the probability of calling position as a DNM in a trio with respect to sequencing depth. DNV rate varies between families and it's somewhat higher for Lithuanian exome data as compared to 1.5×10^{-8} estimated in other research. According to analysis of function annotations we identified 4 possible pathogenic DNM in *MEIS2*, *ULK4*, *DNAH6*, *PGK1* genes. This is the first attempt to estimate DNM rate and constitution for trios from Lithuanian population. This work supported by the LITGEN project (VP1-3.1-ŠMM-07-K-01-013), funded by the European Social Fund under the Global Grant Measure.

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The melatonin receptor 1B polymorphism (rs10830963) might be associated with hyperlipidemia & fatty liver diseases in Korean population

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Introduction: Melatonin Receptor 1B is a receptor for melatonin (the primary hormone secreted by the pineal gland). Several studies suggested the link between disturbances in melatonin production and impaired insulin, glucose, lipid metabolism and antioxidant capacity [1]. Also, genome-wide association studies reported an intronic single nucleotide polymorphism (SNP: rs10830963) as a candidate variant of glucose homeostasis and type 2 diabetes [2–5]. In Korean population study, the rs10830963 was significantly associated with gestational diabetes mellitus [7]. **Materials & Methods:** We tested the association in a Korean population which was recruited by direct-to-consumer genetic predisposition service (GeneStyle-Direct™, TherageneEx Co., Ltd.). From Jul. 2016 to Nov. 2016, total 272 individuals were participated and obtained the written informed consents. We obtained the self-assessment questionnaire which including the items such as birth year, sex, height, weight, waist circumference, and the disease history of hyperlipidemia, type 2 diabetes mellitus, and fatty liver disease. Among the participants, 80% answered the disease history questionnaire. We conducted the chi-square test and logistic regression analysis for disease histories with controlling age, sex, body mass index,

waist circumference. **Results:** The risk allele genotype was significantly enriched in the case groups of all disease histories. Logistic regression analysis showed the risk allele increased the risk of hyperlipidemia (odds ratio = 2.959, p-value = 0.007), Type 2 diabetes mellitus (odds ratio = 5.150, p-value = 0.021), and fatty liver disease (odds ratio = 2.472, p = 0.029). **Conclusion:** Conclusively, we confirmed the association between MTNR1B gene polymorphism and metabolic diseases.

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Ancestry inference and African alleles in the MHC of Canary Islanders

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Introduction: The population inhabiting the Canary Islands (Spain) results from a recent admixture of Europeans (EUR), North Africans (NAF) and sub-Saharan Africans (SSA). Here we evaluated admixture at genomic scale and characterized the ancestry-related mosaic organization of chromosomes (local ancestry) in order to identify regions with larger than average African ancestry.

Materials and Methods: The study was performed on samples from 146 individuals that were genotyped for 580 K variants. Overlapping SNP data from EUR, NAF and SSA from public resources was utilized to estimate both global and local genetic ancestries by means of ADMIXTURE, LAMP-LD and ELAI software. Whole-genome sequencing data ($30 \times$) from a subset of 14 samples were used to study variants with extreme allele frequency differences between EUR and Africans within the identified regions.

Results: ELAI provided better fitting estimates than LAMP-LD based on comparisons against ADMIXTURE estimates. Local ancestry estimates allowed identifying a significant departure from the genome-wide average African ancestry ($|Z\text{-score}| > 3$) in the major histocompatibility complex region. A finer examination of sequence variation in this region validated this observation.

Conclusions: Our results provide a basis for admixture mapping studies in the Canary Islanders and supports the existence of genetic footprints introduced by African

ancestors that can be associated with population disparities in disease traits. Funded by Instituto de Salud Carlos III (FIS PI11/00623 and PI14/00844) and co-financed by the European Regional Development Funds, "A way of making Europe" from the European Union. BGG was supported by a fellowship from ACIISI (TESIS2015010057) co-funded by European Social Fund.

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E-P18.29 Genetic analysis of mitochondrial DNA variants in the population of Pakistan

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Because of its geo strategic position at the crossroad of Asia, Pakistan has gained crucial importance of playing its pivotal role in subsequent human migratory events, both prehistoric and historic. This human movement became possible through an ancient overland network of trails called "The Silk Route" linking Asia Minor, Middle East China, Central Asia, and Southeast Asia. The present study was designed to investigate the whole mitochondrial DNA in 500 unrelated individuals of 22 ethnic groups of the Pakistani population. Sequence analysis revealed, 412 haplotypes in 22 ethnic groups. In spite of that 65% sequences were observed once, 11%, twice, 8% thrice, 5% four time and 2.2% five times. The most common South Asian haplotypes are observed, M 46%, N 7%, and R 13%, while West Eurasian haplotypes are U 18%, H 5%, J 4%, W 3% and T 2% in 22 ethnic groups. A random match probability between two unrelated individuals was found to be 0.01 to 0.06%. While genetic diversity was found to be 0.991 to 0.999, with nucleotide diversity ranging from 0.0089 to 0.0142. The configuration of genetic variation and heterogeneity further unveiled through Multidimensional Scaling and phylogenetic analysis. The results revealed that Pakistani ethnic groups are the composite mosaic of West Eurasian ancestry of numerous geographic origin and they

received substantial gene flow during different invasive movements and have a high element of the Western provenance.

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Are CHRNA5 rs16969968 genotypes associated with nicotine dependence among physicians in Estonia?

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Introduction: The AA genotype (rs16969968) of CHRNA5 gene has been associated with increased risk for nicotine-dependence (ND) and lung cancer. Physicians are well-defined and homogeneous sample, giving the unique opportunity to study genetic architecture of smoking behavior. Therefore, we aimed to evaluate associations between CHRNA5 genotypes and susceptibility to ND among physicians in Estonia. Materials and methods: DNA samples of 823 physicians (218 daily smokers matched by gender and age with 605 non-smokers) were analysed. CHRNA5 rs16969968 genotyping was carried out by using TaqMan Allelic Discrimination Assay. ND was evaluated using The Fagerström Test for Nicotine Dependence (FTND). Chi-squared test and Fisher's exact test were used to compare the distributions of CHRNA5 rs16969968 and FTND. Results: The mean age in both groups was 53 (SD ± 13) years and approximately one third of the physicians were men. The CHRNA5 rs16969968 genotypes were in Hardy-Weinberg equilibrium. The distribution of the genotypes did not differ between smokers and non-smokers: AA (11.5% vs 10.9%), AG (44.0% vs 45.5%) and GG (44.5% vs 43.6%) ($p > 0.05$). The CHRNA5 rs16969968 A allele frequency was similar among smokers and non-smokers - 55.5% vs 56.4% ($p > 0.05$), respectively and no associations of FTND scores (0–5 vs 6–10) with the risk allele and genotypes were found ($p > 0.05$). Conclusions: Based on our results CHRNA5 genotypes are not associated with the nicotine dependence among physicians in Estonia. This study was supported by the Estonian Research Council grant PUT-299.

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E-P18.34**Prevalence of *MC4R*-associated obesity in Slovenian paediatric population**

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Background: The increasing prevalence of morbid obesity is one of the major public healthcare problems worldwide. The environmental and genetic factors involved in the aetiology of obesity are subject of great research interest. Genome-wide association studies identified several genes associated with obesity. Obesity associated genetic variants in melanocortin 4 receptor (*MC4R*) are found in approximately 2% of obese individuals. The aim of this study was to determine the prevalence of *MC4R*-associated obesity in a cohort of obese Slovenian children and adolescents.

Materials and Methods: Promoter and coding regions of *MC4R* gene were analysed in 224 children and adolescents (113 females/111 males; mean BMI-SDS 3.32 ± 0.77 , mean age 13.2 ± 3.9 years) using direct Sanger DNA sequencing.

Results: Eight unique previously reported genetic variants of *MC4R* (NM_005912.2) were identified in 22 participants, of them 2 were classified as pathogenic (c.542 G > A, c.631_634delCTCT), 1 as potentially pathogenic (c.335 C > T), 3 variants of unknown clinical significance (c.*42 T > C, c.*60 C > T, c.-178A > C) and 2 non-pathogenic polymorphisms (c.307 G > A and c.751 A > C). Four participants were carriers of pathogenic *MC4R* variants, including two individuals with the variant c.335 C > T.

Conclusion: The estimated prevalence of *MC4R* associated obesity in Slovenian paediatric population is between 0.9% and 1.8%, warranting the routine analysis of *MC4R* gene in diagnostic protocols of severe obesity. The reported frequency of obesity associated *MC4R* genetic variants is in concordance with previously published reports from other populations.

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E-P18.35**Association study of OPRD1 gene variants with****susceptibility to opioid addiction in an Iranian addicted patients underwent methadone treatment**

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Although methadone is an effective prescribed treatment, its efficacy range is variable significantly among patients which can be explained by genetic variations. OPRD1 is one of the most important genes related to drug dependence, tolerance, reward pathway, hypoxia/ischemia, and mesolimbic dopamine system. We aimed for the first time in Iranian population, to characterize the impact of rs2236857, rs2236855, and rs760589 on occurrence of opioid addiction among patients underwent Methadone Maintenance Treatment. Furthermore, association of these variants with insomnia and libido were investigated for pharmacogenomics aspects. Samples were examined among 202 healthy and 202 opioid addict subjects, all men (20- 60 years old). Genomic DNAs extracted from whole blood samples by Salting Out procedure. The gene variants were genotyped by Allelic discrimination Taqman assay. All analysis was performed by SNPalyze ver.8.1 software. According to the single locus analysis, rs2236857 [P = 0.002, OR = 1.52; 95CI (1.16–1.99)], rs2236855 [P = 0.001, OR = 1.58; 95CI (1.20–2.09)], and rs760589 [P = 0.03, OR = 1.30; 95CI (1.02–1.67)] had significant contributions between groups in the current study, all under a co-dominant hereditary model. By haplotype analysis, four haplotypes were significant in cases and controls. The most common haplotype with overall frequency 36% involved T-C-G (major-major-major alleles) represented highest significance among the study group (P = 3.3E-8). In conclusion, rs2236857, rs2236855, and rs760589, variants of OPRD1, might have a remarkable association with opioid addiction. These variants can be considered as appropriate candidates for pharmacotherapy of MMT and required to be investigated in another populations.

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E-P18.36**Determination of miR-196a Single Nucleotide Polymorphism (SNP) with melting-curve analysis in the population of patients with ovarian cancer**

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Introduction: miRNA molecules are short, non-coding sequences regulating gene expression after transcription. They are important in the development, progression and treatability of tumours. Single Nucleotide Polymorphism (SNP) is the most frequent type of genetic polymorphism. That is true also for miRNAs and their polymorphisms can cause alterations in the gene expression profile. miR-196a was also linked to the genesis of different tumours. Aim: Search for correlation between miR-196a polymorphism and development of ovarian cancer. Materials and Methods: 75 patients with ovarian cancer and 75 healthy persons were investigated. 15–16 mL blood anticoagulated with EDTA was drawn. DNA was isolated with silica absorption method and the melting curve of PCR products generated with LightSnip kit (TibMolbiol, Berlin, Germany) developed for miR-196a (rs11614913) SNP was determined by LightCycler 96 equipment. Allele and genotype frequencies were specified and Student t-test was applied for statistical analysis of data. Results: The Tm of PCR products were 55.5°C for *T* allele and 62.6°C for *C* allele with melting-curve analysis. *T* allele occurred in 32.66% in population of patients and in 40.66% in control group. Genotypes among control persons were 18.66% for *TT*, 44.0% for *TC*, and 37.33% *CC*, while in case of patients these frequencies were 12.0%, 41.33%, and 46.66%, respectively ($p = 0.3815$). Conclusion: miR-196a influences the expression of 684 genes, it requires further complex investigation, whether it is involved in the development of ovarian cancer.

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E-P18.37

Determination of two miR-1936b single nucleotide polymorphism in ovarian cancer patients

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Background: MicroRNAs play important role in the regulation of gene expression. miR-193b (rs1649942; C/T) polymorphism was found to be associated with carboplatin sensitivity in ovarian cancer. Neither rs1649942 nor miR-193b rs30236 (C/T) was not determined in Hungarian ovarian cancer patients yet. Materials and methods: We involved 75 ovarian cancer patients and 75 controls in the study. 15–16 ml EDTA blood was drawn; the DNA was isolated with silica adsorption method. LightSnip kit (TibMolbiol, Berlin, Germany) was used for the determination of rs1649942 and rs30236 polymorphism in the samples. We determined the melting temperatures of the PCR fragments by using LightCycler 96 instrument. We calculated the allele and genotype frequencies and used Student t-test for the statistical calculations. Results: During the study of rs30236 we found T allele in 37.83% in the controls while in 28.28% in the cases, the T allele was present in rs1649942 in 78.00% in the controls and 69.74% in the cancer patients. The genotype analyses did not show significant alteration in the two studied SNPs ($p = 0.1653$ and $p = 0.2626$). Conclusion: We determined the miR-193b rs1649942 and rs30236 SNP polymorphism in a group of Hungarian ovarian cancer patient, but we did not find significant difference in the genotype frequencies comparing the cases and controls. We plan to collect samples from carboplatin resistant patients and make SNP genotyping among them.

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E-P18.38

Clinical presentation of different types of periorbital hyperpigmentation in the presence of polymorphisms in P53 < VEGFA genes

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Introduction: The pathogenesis of periorbital hyperpigmentation (POH) or eye dark-circle is unknown. POH is classified into pigmented, vascular, structural, and mixed type. Evidence exists that link *p53* and *VEGFA* with skin hyperpigmentation and angiogenesis. This study was aimed at identifying the clinical presentations of POH in the presence of *VEGFA* and *TP53* polymorphisms. Materials/methods: A cross-sectional study was conducted among Malaysian-Chinese. Clinical assessments were performed along with collecting medical history and melanin,

erythema index (EI) measurement. Three regions of *p53* and two of *VEGFA* were amplified by PCR followed by direct sequencing using saliva-extracted-DNA. Results: Eighty four participants (28 males, 56 females) were recruited. Family history of POH was prevalent. In majority ($n = 62$) both eyelids were affected. Mixed (pigmentary-vascular) was the most common type. Fourteen polymorphic SNPs were found, eight of them are not reported before. A significant association was observed between CT genotype in *VEGFA* rs3025039 and type of POH ($p = 0.044$), as well as visible vessels in eyelids ($p = 0.035$). Presence of vascular type was significantly associated with *VEGFA* rs699947 ($p = 0.010$). Involvement of both eyelids and homogenous pigmentation was significantly associated with TT genotype in *p53* codon212 ($p = 0.011$). Vascular type was significantly less prevalent among GT carriers in codon217 with skin allergy ($p = 0.036$). Carriers of CC in *VEGFA* rs699947 have significantly higher EI ($p = 0.006$). Conclusion: Polymorphisms in *VEGFA* and *p53* genes were associated with different clinical presentations of POH types. The genetic factors might explain the variability of response to POH treatment.

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E-P18.39

Individuals with hereditary Primary Arrhythmia Syndrome represent A Phenotypic Variability of ANK2 Mutations

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Introduction Alterations in ANK2 have been reported to be the source of assorted arrhythmia phenotypes. The occurrence of ANK2 mutation transporters in hereditary primary arrhythmia syndrome (IPAS), nevertheless, is unidentified in Albanian. By means of a next-generation sequencer, we intended to recognize ANK2 mutations in the cohort of IPAS individuals, in whom conservative Sanger sequencing not succeeded to recognize pathogenic mutations in main contributing genes, and to appraise the clinical features of ANK2 mutation transporters.

Materials and Methods We monitored 524 probands with IPAS and investigated 42 genes counting entire ANK2 exons applying a bench-top NGS (MiSeq, Illumina) or carried out whole-exome-sequencing applying HiSeq2000 (Illumina).

Results 10 of 524 probands (1.8%, aged 0–55 years, 7 males) were discovered to hold 6 dissimilar heterozygous ANK2 mutations. ANK2-W1535R was recognized in 3

LQTS individuals and 1 symptomatic BrS and was calculated as harmful by numerous forecast software. Altogether, as to phenotype, there were 7 LQTS, 1BrS, 2 IVF. Astonishingly, 3/7LQTS patients had the attained category of LQTS (aLQTS). An overall of 6 of 11 individuals had acknowledged malignant ventricular tachyarrhythmia.

Conclusions Assorted ANK2mutations are correlated with an widespread variety of phenotypes, counting aLQTS, particularly with ventricular fibrillation, instead of “ankyrin-B” syndrome.

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E-P18.40

Age of R261* mutation in PAH gene in Karachay-Cherkess Republic (Russia)

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Introduction: Phenylketonuria (PKU) is an inborn metabolic disorder, caused by mutations in the *PAH* gene. That results in decreased metabolism of phenylalanine and severe intellectual disability. The highest frequency of PKU is found on the territory of the Russian Federation in the Karachay-Cherkess Republic (1:850 newborns). This population is characterized by individual *PAH* gene mutation spectrum with major mutation R261*(allele frequency 67,3%, carrier frequency in Karachays 1:16). Materials and Methods: DNA samples of 26 PKU patients with homozygous mutation R261* and 60 healthy Karachays were investigated. STR markers, flanking *PAH* gene D12S1588 (4,82 cM to gene *PAH*), D12S1727 (2,81 cM), D12S78 (1,87 cM), D12S338 (1,87 cM), D12S317 (4,28 cM) were investigated by AFLP method. Results: For each marker linkage disequilibrium was determined and the age of the mutation was calculated. Linkage disequilibrium was $0,58 \pm 0,16$, $0,71 \pm 0,15$, $0,92 \pm 0,08$, $0,93 \pm 0,08$ and $0,73 \pm 0,12$ respectively. In D12S78 and D12S338 linkage disequilibrium was not observed, so we did not consider them. The resulting age by three markers D12S1588, D12S1727 and D12S317 is 11.1, 12.2 and 7.2 generations respectively. The average value of generation number while mutation existed in the population amounted to $10,2 \pm 2,7$. Since the average generation age is 30, we find that the mutation started spreading about 305 ± 80 years ago. Conclusions:

R261* mutation began to spread in Karachays about 100 years before the population growth started (1800s) and further dissemination continued in parallel with

demographic growth. That explains the rapid spread of R261* mutation in Karachays. This work was partially funded by RFBR grants 14-04-00525 and 15-04-01859

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E-P18.42

Obesity increases the prostate cancer risk in Koreans unlike Europeans: a Mendelian randomization Study

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Introduction: Conventionally accepted risk factors of prostate cancer (PCa) are challenged, as large genomic data allows causal inference using Mendelian Randomization analyses. Unlike epidemiologic studies, obesity showed no association in a large Caucasian study. Two recent GWAS on PCa in Asians suggested more similarity than ethnic differences in genetic architecture, which makes the large ethnic gap in PCa more elusive. We attempted to examine the association between PCa and obesity/lipid profiles in Korean men, who still have lower incidence but rapid surge in the PCa rates. **Methods:** We involved 4,122 healthy men from the Korean Genome Epidemiology Study, and 1,001 PCa cases with genetic information. Based on previous GWAS reports, we constructed genetic risk scores (GRS) as genetic instrumental variables (IV) representing BMI, LDL/HDL cholesterol, and triglyceride. Two-stage least square regression analyses were performed to test causal associations. **Results:** The GRS explained 1.15%, 1.85%, 4.03%, and 2.31% of the phenotypic variance for BMI, LDL, HDL, and TG, respectively. All GRS were not pleiotropic to one another (*F*-statistic < 5). GRS for BMI was associated with increased PCa risk (OR per SD increase in BMI-GRS 1.61 [1.20 - 2.17 95% CI]; *p*-value 0.001). All GRS-IV for lipids were not associated with altering PCa risk. **Conclusion:** Our findings suggest obesity per se might be a true risk factor of PCa for Koreans unlike in European descendants, and not through dyslipidemic complications. Whether and to what extent these ethnic differences can explain the current gap in PCa might ensue further studies.

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E-P18.43

Common variant at TCF7L2 gene is associated with increased risk of retinopathy in type 2 diabetic patients

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Diabetic Retinopathy (DR) is a multifactorial disease with a strong genetic component. It classically defined as a microvasculopathy that primarily affects the small blood vessels of the kidney as a complication of diabetes mellitus (DM). Recently, case-control studies demonstrated that the *TCF7L2* (transcription factor 7-like 2 gene) variants were strongly associated With an increased risk of type 2 diabetes. The aim of this study was to investigate the association of three variants at the *TCF7L2* (rs7903146(C/T), rs12255372 (G/T) and rs290487(C/T) with development of retinopathy in an Iranian Population in the north of Iran who have type 2 diabetes (T2D). The genotype and allele analysis of three SNPs were performed in 205 DR and 257 diabetic patients without retinopathy (DNR). Genotypes were determined by using TaqMan technology Allelic discrimination on an ABI7300 system. A significant difference was observed in allele T frequency between DR and DNR subjects in both rs7903146(C/T) and rs290487(C/T). (*P* = 0.0006 and *p* = 0.002 respectively). Also, by genotype frequency comparison, a significant difference were observed under a recessive and dominant genotype models (recessive; *P* = 0.0008, dominant; *P* = 0.02) of rs7903146 (C/T) and rs290487(C/T) variants in DR and DNR individuals. Our results report for the first time the impact of genetic association of the *TCF7L2* variants with progression of retinopathy among T2D patients in an Iranian population in the north of Iran. To substantiate this conclusion, further analysis in a larger sample size and in other Iranian community or Provinces is required.

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E-P18.44

Dissemination of founder mutations among the Druze population in northern Israel

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A number of rare genetic diseases are prevalent among secluded populations of Druze and Arab Moslem origins due to high rates of consanguinity and expansion of founder mutations. Since 2002, specific villages are screened for genetic variations known to cause rare and severe disorders, where carrier frequency is ≥ 1 in 60 in the reference population. Today, this Health Ministry-funded screening includes over 18 rare severe diseases. Summarizing 15 years' experience in screening Galilee and Golan Heights Druze communities, we were able to resolve the diagnosis of multiple patients, each with a unique disease, who were homozygous for the same sequence variant prevalent in heterozygous form in several villages. Mutations causing Cockayne syndrome, Donohue syndrome, and Primary Hyperoxaluria type 1 (PH-1) are three such examples. Interestingly, the genetic variation that underlies Prolidase deficiency (p.Ser202Phe in *PEPD*) and the genetic variation that causes PH-1 (c.33_34delC in *AGXT*) were prevalent in both Moslem and Druze villages. Over the last decade, we have witnessed dramatic changes in attitudes toward genetic screening in the four non-Jewish sectors in the region we serve and survey. Furthermore, consanguinity rates in the Galilee and Golan Heights have dropped in recent years, indicating changes in traditional customs that may lead to broader acceptance of inter-village marriages, which may result in dissemination of founder Druze mutations across different villages. Thus, it is essential to assess the establishment of a central screening test for the identification of couples at risk for affected babies among the entire Druze population.

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E-P18.45 Genomewide association study of facial temperature before and after washing

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Skin temperature determined by the heats from internal metabolic process and/or outside environments. The facial skin temperature was greatly differed among individuals and between before and after washing the face. We assumed that the skin temperature differences are depends on their cosmetics, UV exposure, and genetic factors related to the underlying metabolic pathways. We recruited about 500 participants who visited to a cosmetic laboratory of AMORE PACIFIC Co., Ltd. All the participants confirmed the written consents, and this research approved the

institutional review boards. Each participants was measured the facial temperature by infrared camera. Average facial temperatures for the before and after washing face, respectively, were used the genomewide association studies. The genetic information was obtained by precision medicine research array of Affymetrix. Total 900k SNPs were used to test the GWAS, and we could identify three suggestive SNPs (rs4548934, rs142551041, rs7052636) for the facial temperature before washing, and two suggestive SNPs (rs9859074, rs4350340). The markers can be used for the prediction of facial temperature after cosmetic make up, and can be used for guiding the personalized make up. We hope this results might be tested in independent population. This research supported by the AMORE PACIFIC and THERAGEN ETEX companies, and there is no conflict of interests.

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E-P18.48

Familial Amyloid Polyneuropathy (FAP ATTRV30M): survival analysis confirms gender differences in early-onset (< 40) but not in late-onset patients (> 40)

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Introduction: FAP (ATTRV30M) is an autosomal dominant systemic amyloidosis due to a point mutation in *TTR* gene. First described in Portugal by Andrade (1952), Portuguese patients have been characterized by early onset (35.4 yrs). However, AO now ranges 19–82 yrs and asymptomatic carriers aged > 90 yrs are known to have children with late and early-onset. **Materials and Methods:** From the data registry at UCA, we retrieved data on 2713 patients (1461 men) and 604 asymptomatic carriers (212 men) on regular follow-up: gender, AO or age-at-last examination (ALE for asymptomatic carriers). We used t and Mann-Whitney tests. Survival curves for AO (ALE of asymptomatic carriers included as censored data) were estimated by Kaplan-Meyer methods and compared by gender using a log-rank test. Statistical analysis used SPSS v.23. **Results:** mean AO was significantly different in men (33.4 y) and women (37.6 y), ($p < 0.001$); in the asymptomatic group no gender differences were found for mean ALE (38.0 vs. 38.6). In the early-onset group women had later onset (32 y) than men (29 y) ($p < 0.001$), while in the

late-onset it was 53 y (women) and 57 y (men) ($p < 0.005$). With survival analysis, overall gender distributions were different, as well as when AO < 40 yrs; however, when AO ≥ 40 yrs, no significant differences were found.

Conclusions: probably due to a pool of older asymptomatic women, gender differences were no longer apparent for AO ≥ 40, whereas conventional tests showed gender differences in opposite directions in the groups with AO < 40 and with AO ≥ 40.

Next we should explore whether we have more than one underlying distribution for each gender.

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E-P18.49

Association study of genetic variations in CD1A, CD1D and risk of tuberculosis in an Iranian Population

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Introduction: Pulmonary tuberculosis (PTB) caused by Mycobacterium tuberculosis (MTB), is still a public health concern, and leading cause of morbidity and mortality

throughout the world especially in Africa and Asia. Cluster of differentiation 1 (CD1) is a family of glycoprotein which expressed on antigen presenting cells (APC). They are belongs to major histocompatibility complex class I (MHC) and participate in presentation of lipid antigens to T cells. The current study aimed to examine the possible association between CD1A and CD1D polymorphisms, and risk of pulmonary tuberculosis (PTB) in an Iranian population.

Material and Methods: This case-control study was conducted on 172 PTB patients and 180 healthy subjects. The CD1A rs411089, CD1A rs366316, CD1D rs973742, CD1D rs859009, CD1D rs859010 polymorphism were genotyped using PCR-RFLP.

Results: The results demonstrated that CC genotype of CD1A rs411089 polymorphism in recessive model increased the risk of PTB in comparison with TT + TC genotypes ($OR = 2.71$, 95% CI = 1.36–5.40, $P = 0.004$). GC genotype as well as GC + CC genotype of CD1D rs859009 Polymorphism decreased the risk of PTB ($OR = 0.49$, 95% CI = 0.29–0.85, $P = 0.01$ and $OR = 0.53$, 95% CI = 0.31–0.89, $P = 0.01$, respectively).

Conclusion: Our results proposed that CD1A rs411089 polymorphism may increase the risk of PTB, but CD1D rs859009 Polymorphism decreased the risk of PTB. Furthers studies with larger sample sizes and different ethnicities are necessary to confirm our findings.

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E-P18.50

FarGen: The Faroese Reference Genome and Demographic Inference of the Faroese population

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Introduction: The FarGen-infrastructure is an ongoing government funded project that aims to sequence the whole-genome of individuals from the isolated population of the Faroe Islands (www.fargen.fo). Using FarGen data we aim to construct a whole-genome data set for detection of population-specific genetic variants, indels and rearrangements, novel, rare and *de novo* variants as well as

haplotypes. Additionally, we will construct a high-quality whole-exome data set for demographic inference of the Faroese population. Materials and Methods: From the 1500 individuals recruited to the FarGen-infrastructure we will select 24 healthy trios in addition to 600 healthy single-individuals, representing six sub-regions of the archipelago. The trio-lineages will be verified by the national-genealogy registry at the Genetic Biobank (www.genetics.gov.fo). The 72 genomes and 600 exomes will be sequenced using Illumina technology and aligned to the human reference genome. Further data processing will be performed using the Genome Analysis Toolkit, in addition to various statistical packages for *de novo* variants detection and genetic stratification measurement. Expected results and perspectives: The two data sets will establish a standard of comparison in identification of disease-associated genes. Additionally, the genomes will provide a haplotype-resolved resource for downstream imputation of genome-wide association data, as well as the large number of exomes will permit an evaluation of the degree of population stratification in the Faroese population. Overall, this unique resource will enable a population-wide study of how genes, environment, and lifestyle affect the health of an entire nation. Funding: The project is funded by the Danish and Faroese Governments.

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E-P18.51

Haplotype analysis for five unrelated patients with a novel *ATP7B* gene mutation

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Abstract Introduction: Wilson disease which is a rare autosomal recessive disorder of copper metabolism is due to the disease causing mutations in the *ATP7B* gene. With the use of combination of markers such as SNPs on a single chromosome, it is possible to determine the structure of haplotype in the human genome, in which can provide notable information regarding the origin of the mutation in human genetic disorders. Since we found a novel mutation in exon 18 of *ATP7B* gene in five unrelated patients affected by Wilson disease, the purpose of this study was to determine a SNP haplotype analysis for these families and also 48 unrelated individuals to show whether they have the same origin.

Materials and Methods: DNA was prepared from these cases and then PCR was performed for four regions covering four SNPs with higher frequency in our population which included c.1216 T > G (exon2), c.1366 G > C (exon 3), c.3903 C > T (18), c.4021 + 50 G > C (19). These SNPs are located in upstream and downstream of this novel mutation. Then, the PCR products were subjected to DNA sequencing using both forward and reverse primers (Eurofins Genomics, Germany).

Results: 3 different haplotypes were found in the present study and the patients with the same mutation had the same haplotype (T-G-T-C). The most prevalent haplotype in this study was G-C-T-C.

Conclusion: Since these 5 geographically separated families with the same mutation had the same haplotype, we concluded that this mutation possibly had the same origin in this population.

S. Ghashghaei: None.

E-P18.52

The genetic legacy of Zoroastrianism in Iran and India: Insights into population structure, gene flow and selection

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The origins of Zoroastrianism, one of the oldest extant religions in the world, is usually attributed to the teaching of the Iranian prophet Zoroaster around 1200 BCE. According to tradition, after the Muslim conquest of Persia (present day Iran) in the 7th century CE, some Zoroastrians sailed to India to escape persecution. The date of their arrival in India is unknown; traditional sources place it variously between 716 and 936 CE. We explore the genetic legacy of Zoroastrianism in detail using novel genome-wide autosomal (Affymetrix Human Origins SNP array) and Y/mtDNA genotype data for Iranian and Indian Zoroastrian individuals. By comparing these with other publicly available genetic data, and exploiting linkage disequilibrium information in the autosomal genome, we infer the demographic processes, including the extent, timing and sex-specific components of migration, admixture and isolation, that have contributed most to the genetic landscape of modern Zoroastrians. In addition, we assess genetic support for claims of patrilineal recent common ancestry among Parsi priests. Finally, we identify candidate genomic regions under positive selection in Zoroastrian populations, which may relate to the prevalence of diseases or distinct phenotypic traits in the community.

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E-P19 Genetic counselling/Education/public services

E-P19.03

How does genetic counselling help coping with male infertility and unexpected findings?

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Introduction: We present the case of a man with 3 years primary infertility, diagnosed with azoospermia. The man was referred to the Genetic Department during his fertility investigation. **Materials and Methods:** Karyotypic analysis, Chromosome Y microdeletion analyses and proper Genetic Consultation. **Results:** Genetic analysis revealed (i) mosaicism 45,XO/46,X der(Y) and (ii) complete deletion of AZFb + c regions (sY121, sY127, sY134, sY254, sY255 markers deleted). These findings are in agreement with published data supporting that Yq microdeletions may cause Y chromosome instability, leading to 45,XO cell lines. Men with this genetic profile should be discouraged considering a TESE/ICSI program, as presence of testicular spermatozoa has never been reported. In our case, “bad news” was not an option for our patient, as anxiety, isolation, fear, emptiness, insecurity, guilty, shame and personal failure were his feelings. Disbelief and denial are common symptoms experienced in men coping with infertility. Genetic consultation and diagnosis are the key starting points before decision making. With active listening, open questions and encouragement by the counsellor, the man started to search “out of the TESE-option box” for effective alternatives, such as sperm donation or adoption. Answering questions about sexual life and ability, personal image, marriage and relation was also important part of the counselor’s task, enabling patient to feel supported through the process and make effective choices, fitted at their life style and social background. Finally, as mosaic events have higher risk for developing cancer, health screening recommendations were provided. **Conclusion:** Genetic Counselling help effectively to deal unexpected findings related to azoospermia.

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E-P19.04

Evidence of germline mosaicism in Hemophilia A: implication of genetic counseling

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Hemophilia A is an inherited X-linked disorder. This study reports a family with an affected hemophilia boy in

which germline mosaicism in the mother complicates the X-linked inheritance of the disease. There was no family history of hemophilia in the family. It seems that denovo mutation or germline mosaicism could be responsible for the disease occurrence. Direct sequencing of FVIII gene in proband revealed c.3637delA (p.I1213>Ffs5) mutation in the 14th exon. Direct genetic testing of the mother showed no mutation. Additionally, we could track the mutated allele using linkage analysis with the help of STR (Short Tandem Repeat) markers linked to the FVIII gene. Haplotype analysis as an indirect strategy showed his mother was a carrier. One of his sisters was normal with direct mutation analysis but haplotype mapping suggested her as a carrier. The second sister was normal with the direct and indirect approaches. The third one was carrier with both methodologies.

Based on the obtained results, one possible mechanism could be germline mosaicism of the mother. Gonadal mosaicism usually occurs in females and can complicate data analysis. DNA testing may help carrier detection but negative results will not rule out the possibility of mosaicism. This observation suggests the importance of confirming the carrier status of the family members with different strategies. Since mosaicism after having an affected child is consistent with gonadal/somatic mosaicism, the recurrence risk is significantly increased. The results have important implication in genetic counseling in X-linked disorders.

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E-P19.05 Psychosocial issues of Filipino parents with a child with Maple Syrup Urine Disease

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Introduction: Maple Syrup Urine Disease (MSUD) is a common inborn error of metabolism diagnosed in the Philippines. A family may experience stress, anxiety, sorrow or feelings of helplessness when a child is diagnosed to have a genetic disorder which can lead to chronic care and disability. An illness in a child affects not only the child, but the whole family as well. **Materials and Methods:** In-depth interviews using a semi-structured set

of questions was done between the months of November 2015 – March 2016. A total of 12 parents were interviewed. **Results:** The diagnosis of MSUD in a child is indeed a stressful event for the family. Parents experienced fear, confusion, and hurt, among other emotions. Having a child with MSUD had a negative impact on their families, especially in terms of financial burden, dietary restriction, and marital conflicts leading to separation. However, some parents reported positive effects such as increased confidence in one's abilities to care for the affected child and closer relationships among family members. **Conclusion:** The findings of this study reflect the complex issues and problems of families with a child affected by MSUD. It will help form policies and guidelines for genetic counseling of MSUD patients and their families in the Philippines to assist families in coping with the diagnosis and improve outcomes of affected children.

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E-P19.06 Precision medicine and the need for generic risk literacy

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With the advent of precision medicine and the so-called mainstreaming of genetics in healthcare, genetic testing results are increasingly used outside the context of clinical genetics. One of the implications is that many health care workers who have little or no experience or training in interpreting and explaining genetic test results, have to communicate genetic findings to their patients.

Moreover, the genetic tests that are currently being marketed tend to include more and more moderate and intermediate risk factors. As a consequence, the risks connected to these genetic test results are in shades of grey and need to be weighted and communicated cautiously. Not only for precision purposes, but also for the purpose of genetic counseling, an aspect that is inseparably linked to communication genetic testing results.

There is much attention in literature regarding the need to improve genomic literacy among health care workers and the general public. In this contribution however, we will focus on the need to improve *genetic risk literacy* in order to facilitate adequate counseling. Which is the role that we as geneticists, both trained and experienced in genetic risk communication, could/should play in educating colleagues and the general public? How can we help making genetic

risk understandable based on the experiences we have in the field of clinical genetics.

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E-P19.07

Creativity in writing essays when learning medical genetics

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Teaching for long-term, so that physicians could easily understand new developments in genetics, even if it is not their specialty, calls for achieving the highest level of Bloom's revised taxonomy. In this study the objective was to investigate students' opinion on their own creativity. First year medical students of the Carol Davila Medicine and Pharmacy University were asked during their training in genetics to write an essay. Afterwards they were given a questionnaire, to state if they felt creative or not while writing the essay; and also to choose from a five level scale if they enjoyed the task and gathering information for it, if they wrote something original and if it was easy or difficult to do so. The 6 questions of the questionnaire were answered by 88 students. The results showed that 41 students felt creative while writing the essay. 19.32% enjoyed (much and very much) writing it, 11.36% did not at all like the task and 40.91% chose as answer 'intermediate'. 37.5% enjoyed gathering data for conceiving the essay, while 38.64% were equidistant when they answered this question. When asked if their essay was original 29.55% of students chose 'enough' and 32.95% were equidistant by answering 'intermediate'. Although some of the students had a clear-cut opinion, still most of them preferred to be equidistant. Despite the small sample and other biases the study proved the necessity of teaching students to firmly state their

opinion and also of asking students to write essays on subjects they select.

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E-P20 Psychological/Ethical/legal issues

E-P20.02

Health-related quality of life and disability in patients with haemophilia

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Background: EQ-5D is one of the instruments used for the evaluation of life quality in patients chronic diseases. The aim of the study is evaluation of life quality in haemophilic patients.

Methods: The study included a number of 52 patients with severe A and B haemophilia, all of them with chronic haemophilic arthropathy, aged between 16–45, submitted to EQ-5D questionnaire. The articular orthopedic score was correlated to VAS scale (Pearson-r parameter). The results were examined on the whole group and on age groups.

Findings: Our results revealed that haemophilia severity had influence on self-care domain ($p = 0.338$) while patients age impacted mobility ($p < 0.001$) and usual activities ($p = 0.003$). We observed significant differences in mobility ($p = 0.041$), pain ($p = 0.007$), usual activities ($p = 0.027$) domains. The VAS scale reveals values significantly reduced in patients from older age groups as compared with the age group 16–24 years old ($p = 0.002$).

Discussion: As haemophilic patients with severe form and chronic haemophilic arthropathy grow older, limitation of daily routine due to pain and mobility damage determine a significant decrease in life quality.

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